PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 23 November 2000 (23.11.00)	
International application No. PCT/EP00/03100	**Applicant's or agent's file reference** SCB540PCT
International filing date (day/month/year) 07 April 2000 (07.04.00)	**Priority date** (day/month/year) 09 April 1999 (09.04.99)
Applicant COLACCI, Annamaria et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

03 November 2000 (03.11.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

Charlotte ENGER

Telephone No.: (41-22) 338.83.38

On the other hand, such potential chemopreventive effect does not allow to draw conclusions about any potential antimetastatic effect of lipoic acid. This effect does not depend on cytotoxic or cytostatic mechanisms, but rather involves the inhibition of cell migration, adhesion and invasion.

DEFINITION OF THE USED WORDS

The following definitions will be used in the disclosure of the invention.

Invasiveness: Ability of cells to cross anatomic barriers, such as basal membranes, interstitial stroma and intercellular junctions which divide tissue compartments (Mignatti and Rifkin, Physiol. Rev., 73: 161-195, 1993).

Migration: one of the steps of invasion, motility, which allows tumour cells to cross basal membrane and stroma (Liotta et al., Sem. Cancer Biol., 2: 111-114, 1991).

Chemoinvasion: Invasive response of the cells to a chemoattractant stimulus.

Chemoattractant: mixture of substances of cellular derivation capable of stimulating directional migration.

Adhesion: ability of the cells to specifically recognise and attach to extra-cellular matrix.

DISCLOSURE OF THE INVENTION

It has surprisingly been found that alpha lipoic acid (LA) or the salts thereof have a high antimetastatic activity at micromolar doses; lipoic acid inhibits chemoinvasion and causes an increase in tumour cell adhesion to the extra-cellular matrix. Alpha lipoic acid can be used either as the racemate or in the enantiomerically pure form.

The antimetastatic activity of lipoic acid was demonstrated by using a chemoinvasion model (Albini et al., Cancer Res., 47: 3239-3245, 1987; Reich et al., In:

"Alternative Methods in Toxicology, Goldberg and Liebert eds., Vol. 7, pp 11-22, 1989), which allows a rapid, quantitative and reproducible assessment of the invasive and metastatic potential of malignant cells and therefore a reliable identification of molecules with antimetastatic activity. In vitro models mimicking the invasion process are effective screening tools to detect for detecting compounds with antiinvasive and antimetastatic activities (Hart and Fidler, Cancer Res., 38: 3218-3224, 1978; Liotta et al., Cancer Lett., 11:141-147, 1980; Starkey et al., Cancer Res. 44: 1585-1594, 1984, Mareel et al., Inv. Met. 1: 195-204, 1981). Further evidences of the antimetastatic activity of lipoic acid are provided by its high ability to promote cellular adhesion to the basal membrane. Again, a standard in vitro protocol was used (Kato and De Luca, Exp. Cell Research 173, 450-462, 1987; Kato et al., Exp. Cell Research 179, 31-41, 1988; Kim et al., Inv. Met 14, 1-6: 147-155, 1994-1995).

EXPERIMENTAL SECTION

The cell lines used in this test show a fully malignant phenotype: murine fibroblasts (BALB/c 3T3) transformed with carcinogenic agents: 1,2-dibromoethane (clone F4), 3-methylcholanthrene (clone MCA1), benzo(a)pyrene (B(a)P); murine fibroblasts (NIH3T3) transfected with H-ras (NIH/ras), and the human fibrosarcoma cell line HT1080.

CHEMOINVASION ASSAY

The chemoinvasion assay was performed according to the standard procedure (Albini et al., Cancer Res., 47: 3239-3245, 1987; Melchiori et al., Inv. Met., 12, 1-12, 1992, Adatia et al., Inv. Met., 13: 234-243, 1993; Albini, Pathol. Oncol. Res. 4, 3: 230-241,1998) using the artificial basal membrane Matrigel(R). In the chemoinvasion assay, normal fibroblasts and epithelial cells, as well as

cells deriving from benign tumours, cannot cross the Matrigel(R) coating. Malignant cells, having specific basal membrane degrading enzymes, penetrate the gel and migrate to the lower surface of the filter after 6 hour incubation. The number of metastatic cells crossing the Matrigel(R) and their malignant behaviour are directly related (Albini et al., Cancer Res. 47: 3239-3245, 1987).

The following Tables 1-3 show the number of cells (per field) which crossed the Matrigel(R) barrier and the percentage of invasion inhibition compared to simultaneously tested controls. The mean of three different experiments in triplicate are reported. A reduction of invasion $\geq$30% is considered to be significant (Welch et al., Int. J. Cancer :43, 449-457, 1989).

Table 1 shows results from the assay performed pre-treating the malignant cells with alpha lipoic acid. 70% confluence cells were treated with an alpha lipoic acid solution (0.1-100 μM) obtained by dissolving the product in 1N NaOH. After 16h the cells were harvested with trypsin-EDTA (0.05% and 0.02%, respectively), resuspended in 10% NCS D-MEM, centrifuged, washed with D-MEM containing bovine serum albumin (BSA, 0.1%), centrifuged again and resuspended in the same medium. The viability and the number of cells were assessed by the trypan blue exclusion test. The invasion assay was performed according to the standard procedure (Albini et al., Cancer Res. 47: 3239-3245, 1987) using a cell suspension containing 1.5 x 10^5 cells / chemotaxis chamber.

Table 1. Effects of LA pretreatment (16 hrs) on the invasive behaviour of murine cells transformed by chemicals or by oncogene transfection.

	DBE/F4		MCA1		B(a)P		NIH/ras	
LA (μM)	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition
0	116 ± 3		116 ± 2		151 ± 4		174 ± 6	
0.1	78 ± 1	32 ± 1	77 ± 1	39 ± 1	140 ± 1	7 ± 1	134 ± 5	23 ± 1
1	60 ± 1	48 ± 1	60 ± 1	48 ± 1	109 ± 2	28 ± 1	102 ± 2	41 ± 1
10	48 ± 1	28 ± 1	40 ± 1	65 ± 1	86 ± 3	43 ± 2	74 ± 3	57 ± 1
100	42 ± 1	64 ± 1	30 ± 1	74 ± 1	68 ± 1	55 ± 1	62 ± 10	64 ± 6

In table 2 results of an invasion assay performed in the presence of alpha lipoic acid are reported. Exponentially growing cells were harvested with trypsin-EDTA (0.05% and 0.02% respectively), resuspended in 10% NCS D-MEM, centrifuged, washed with D-MEM containing bovine serum albumin (BSA, 0.1%), centrifuged again and resuspended in the same medium containing alpha lipoic acid (0.1-100 μM conc.) previously solubilised in 1N NaOH. The viability and the number of cells were assessed by the trypan blue exclusion assay. The invasion assay was performed according to the standard procedure (Albini et al., Cancer Res. 47: 3239-3245, 1987) using a cell suspension containing 1.5 x 10^5 cells/ chemotaxis chamber (0.8 ml).

Table 2. Effects of LA treatment on the invasive behaviour of murine cells transformed by chemicals or by oncogene transfection.

	DBE/F4		MCA1		BP		NIH/ras	
LA (μM)	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition
0	120 ± 1		102 ± 2		154 ± 2		130 ± 2	
0.1	85 ± 1	29 ± 1	80 ± 1	21 ± 1	105 ± 4	32 ± 1	73 ± 1	44 ± 1
1	78 ± 1	35 ± 1	72 ± 2	40 ± 2	106 ± 1	31 ± 1	67 ± 2	48 ± 2
10	44 ± 1	63 ± 1	47 ± 1	61 ± 1	85 ± 3	45 ± 2	56 ± 1	57 ± 1
100	40 ± 1	67 ± 1	35 ± 1	66 ± 1	72 ± 1	53 ± 1	37 ± 1	71 ± 1

Table 3 shows the results from the invasion assay carried out on HT1080 cells. This cell line was isolated from a human fibrosarcoma and is widely used in cancer research because of its characteristics (high invasive and metastatic behaviour).

70% confluence cells were pre-treated with a solution (0.1-100 μM) of alpha lipoic acid (obtained by dissolving the product in 1N NaOH), or were resuspended after removal in 10% NCS D-MEM medium containing alpha lipoic acid (0.1-100 μM). The assay was carried out according to the standard procedure (Albini et al., Cancer Res. 47: 3239-3245, 1987) using a cell suspension containing 1.5 x 10^5 cells / chemotaxis chamber.

Table 3. Effects of LA on the invasive behaviour of HT1080 cells

	Pretreatment		Simultaneous treatment	
LA (μM)	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition
0	434 ± 2		438 ± 2	
0.1	391 ± 1	10 ± 1	387 ± 5	12 ± 1
1	323 ± 13	26 ± 3	311 ± 1	29 ± 1
10	311 ± 3	28 ± 1	249 ± 4	43 ± 1
100	198 ± 2	54 ± 1	188 ± 4	57 ± 1

RESULTS OF THE CHEMOINVASION ASSAY

The results clearly demonstrated the anti-invasive dose-related effect of lipoic acid. The compound inhibits the invasive capability of malignant murine cells obtained by chemical transformation (clones MCA1 and DBE/F4) or by transfection with an activated oncogene (NIH/ras). Consistent results are obtained in human fibrosarcoma - derived cells. Compared with the untreated control, a 30% inhibition is observed at dosages ranging from 0.1-1μM. Similar results are observed when dissolving alpha lipoic

acid in KOH, tris(hydroxymethyl)-aminomethane or EtOH. The micromolar activity as well as the lack of toxicity demonstrate that lipoic acid strongly inhibits the invasion process and its effect does not depend on the administration schedule.

ADHESION ASSAY

Cell adhesion, a basic phenomenon for the metastatic process, was tested through of a widely used in vitro model (Kato and De Luca, Exp. Cell Research 173, 450-462, 1987; Kato et al., Exp. Cell Research 179, 31-41, 1988; Kim et al., Inv. Met 14, 1-6: 147-155, 1994-1995). Adhesion to the extracellular matrix plays a pivotal role in assessing the ability of tumour cells to migrate to distant sites, leading to metastasis onset. A high adhesion to the extracellular matrix is linked to a lower tendency to migrate (Wagner et al., Proc. Natl. Acad. Sci. USA 92: 7411-7415, 1981; Varner and Cheresh, Curr. Opin. Cell Biol. 8: 724-730. 1996) and therefore to an antimetastatic effect of the product (Glinsky, Cancer and Met. Rev., 17: 177-185, 1998).

Exponentially growing cells were mechanically removed, resuspended in 0.05% BSA D-MEM and centrifuged twice. The cell number was evaluated by Trypan-blue exclusion assay and then diluted with 0.05% BSA D-MEM containing lipoic acid (100-500 μM) to a density of 2×10^5 cells / ml, 1 ml of cell suspension per plate and incubated for 2 h at 37°C 5% CO_2. Plates were coated according to the procedure previously described (Kato and De Luca, Exp. Cell Research 173, 450-462, 1987; Kato et al., Exp. Cell Research 179, 31-41, 1988; Kim et al., Inv. Met 14, 1-6: 147-155, 1994-1995) using as adhesion substrates fibronectin (3 μg/ml), laminin (10 μg/ml), vitronectin (3 μg/ml max conc.), collagen IV (10 μg/ml). Plates were then washed 3 times with adhesion medium, washed with PBS, fixed and stained in 0.2% crystal violet in 20% methanol for 10 min. The excess

of dye was removed.

Tables 4 - 7 show the optical density (measured at 560 nm wavelength) of the solution obtained by solubilising the dye fixed to the cells with 1% SDS. Optical density is therefore directly related to the number of cells attached to the substrate after the incubation time (2 hours). The data reported in the tables are the means of three different experiments in triplicate.

Table 4. Effects of LA on adhesion of murine transformed cells on laminin (10 µg/ml).

LA (µM)	MCA-1 (O.D. ± S.E.)	DBE/F4 (O.D. ± S.E.)	B(a)P (O.D. ± S.E.)
0	0.284 ± 0.020	0.286 ± 0.008	0.070 ± 0.007
100	0.262 ± 0.002	0.451 ± 0.038	0.070 ± 0.006
250	0.314 ± 0.041	0.481 ± 0.007	0.147 ± 0.004
500	0.468 ± 0.034	0.961 ± 0.116	0.173 ± 0.014

Table 5. Effects of LA on adhesion of murine transformed cells on type IV collagen (10 µg/ml).

LA (µM)	MCA-1 (O.D. ± S.E.)	DBE/F4 (O.D. ± S.E.)	B(a)P (O.D. ± S.E.)
0	0.121 ± 0.010	0.180 ± 0.004	0.135 ± 0.022
100	0.156 ± 0.015	0.221 ± 0.014	0.251 ± 0.028
250	0.172 ± 0.009	0.237 ± 0.035	0.299 ± 0.025
500	0.328 ± 0.001	0.473 ± 0.09	0.575 ± 0.026

Table 6. Effects of LA on adhesion of murine transformed cells on fibronectin (3 µg/ml)

LA (µM)	MCA-1 (O.D. ± S.E.)	DBE/F4 (O.D. ± S.E.)	B(a)P (O.D. ± S.E.)
0	1.115 ± 0.035	0.559 ± 0.048	0.476 ± 0.014
100	1.176 ± 0.019	0.614 ± 0.079	0.562 ± 0.009
250	1.153 ± 0.025	0.734 ± 0.048	0.561 ± 0.027
500	1.344 ± 0.025	0.944 ± 0.010	0.728 ± 0.004

Table 7. Effects of LA on adhesion of murine transformed cells on vitronectin (3 µg/ml)

LA (µM)	MCA-1 (O.D. ± S.E.)	DBE/F4 (O.D. ± S.E.)	B(a)P (O.D. ± S.E.)
0	1.658 ± 0.045	1.400 ± 0.053	0.877 ± 0.016
100	1.630 ± 0.100	1.434 ± 0.028	1.026 ± 0.043
250	2.292 ± 0.072	1.732 ± 0.018	1.061 ± 0.003
500	2.415 ± 0.030	2.199 ± 0.042	1.082 ± 0.043

RESULTS OF THE ADHESION ASSAY

Alpha lipoic acid induces a reduction in cell migration while enhancing adhesion to the matrix. In fact, a 500 µM concentration induces an about 2.5 times increase in adhesion to laminin and to collagen IV, and an about 1.5 times increase in adhesion to fibronectin and to vitronectin.

What stated above clearly evidence that lipoic acid or the physiologically equivalents analogues thereof (salts, esters, solvates, inclusion complexes and the like) can advantageously be used for the preparation of antimetastatic drugs.

For the scheduled therapeutical uses, lipoic acid can be administered through the oral, intravenous (US 5569670), subcutaneous (WO97/10808) routes or through the other conventional administration routes (topical, inhalatory, rectal, etc.).

Because of the extremely low toxicity of lipoic acid, it can therefore be administered at very high doses.

The possibility to carry out chronic oral administrations is of course a remarkable advantage of the present invention.

As a rule, daily posology will range from about 0.5 to about 5 g, optionally subdivided in repeated administrations, depending on the disease and the conditions of the patient (weight, sex and age).

Suitable formulations of lipoic acid can be prepared according to conventional techniques.

CLAIMS

1. The use of alpha lipoic acid or physiologically equivalent derivatives thereof for the preparation of antimetastatic medicaments.

2. The use as claimed in claim 1 wherein the physiologically equivalents derivatives of lipoic acid are selected from salts, esters or inclusion complexes.

3. The use as claimed in claim 2 wherein the lipoic acid derivative is a pharmaceutically acceptable salt.

4. The use as claimed in any one of claims 1-3 for the preparation of antimetastatic medicaments which can be administered through the oral, intravenous or subcutaneous routes.

INTERNATIONAL SEARCH REPORT

Ir. [redacted] Application No
PCT/[redacted] 00/03100

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/385 A61P35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE, WPI Data, PAJ, EMBASE, BIOSIS, CANCERLIT, AIDSLINE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLACCI A ET AL: "Inhibition of chemically induced cell transformation by lipoic acid (Meeting abstract)." PROC ANNU MEET AM ASSOC CANCER RES, (1997). VOL. 38, PP. A2419. ISSN: 0197-016X., XP000929597 Istituto Nazionale per la Ricerca sul Cancro (IST) Biotechnology Satellit Unit, Bologna, Italy 40126. the whole document	1,4

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2000	31/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Cielen, E

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SILINGARDI P; NOONAN D; HORN W; VACCARI M; ARGNANI A; GRILLI S; IACONDINI A; COLACCI A : "Effect of lipoic acid on foci forming capacities of transformed cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 39, March 1998 (1998-03), pages 289-290, XP000929579 cited in the application the whole document	1
X	US 5 679 697 A (GARNETT MERRILL) 21 October 1997 (1997-10-21) abstract column 1, line 10 - line 18 column 4, line 43 - line 65 column 5, line 14 - line 40 column 12, line 33 - line 41 column 13, line 58 -column 14, line 25 column 14, line 65 -column 15, line 19 column 15, line 59 -column 16, line 3 claims	1-4
X	WO 99 06040 A (BERRY CHRISTOPHER J ;PACKER LESTER (US); FOLEY JOHN L (US)) 11 February 1999 (1999-02-11) abstract page 1, line 5 - line 9 page 10, line 13 - line 23 page 13, line 12 - line 16 page 15, line 24 - line 28 page 16, line 26 -page 17, line 2 page 17, line 25 -page 18, line 4 page 19, line 9 - line 14 page 30, line 7 - line 13 claim 1	1,2,4
X	WO 95 13061 A (IMMUNAL KFT ;KULCSAR GYULA (HU)) 18 May 1995 (1995-05-18) abstract page 3, line 13 - line 21 page 4, line 10 - line 20 page 5, line 1 - line 29 page 6, line 1 - line 5 page 16 page 22, line 1 - line 5 claims 1,4,7,9,16,18-30	1-4

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INTERNATIONAL SEARCH REPORT

[redacted] Application No
PCT/[redacted] 00/03100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 36773 A (UNIV YALE) 27 August 1998 (1998-08-27) abstract page 3, line 16 -page 4, line 3 page 4, line 19 - line 27 page 5, line 6 - line 9 page 13, line 1 - line 9 page 15, line 19 -page 16, line 6 page 23, line 24 - line 28 page 25, line 20 - line 23 table 1 claims	1-4
X	CH 683 920 A (MARIGEN SA) 15 June 1994 (1994-06-15) cited in the application abstract page 2, line 1 - line 30 page 4, line 60 -page 5, line 8 page 5, line 41 - line 44 page 15, line 54 - line 56 page 16, line 13 - line 27 page 21, line 30 - line 65 claims	1,2,4
E	WO 00 24734 A (UNIV NEW YORK) 4 May 2000 (2000-05-04) abstract page 1, line 10 - line 16 page 5, line 1 - line 25 page 6, line 19 - line 35 page 15, line 9 - line 32 page 17, line 29 -page 18, line 2 page 19, line 5 - line 20	1-4

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PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

PCT/EP 00 / 03100

International Application No.

07 APR 2000

International Filing Date

(07. 04. 2000)

EUROPEAN PATENT OFFICE
PCT INTERNATIONAL APPLICATION

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* SCB540PCT

Box No. I TITLE OF INVENTION THE USE OF ALPHA LIPOIC ACID IN THE ANTIMETASTATIC TREATMENT

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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This person is:

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☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

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Continuation of Box No. III FU[illegible]ER APPLICANT(S) AND/OR (FURTHER) IN[illegible]TOR(S)

If none of the following sub-boxes is used, this sheet should not be included in the request.

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State *(that is, country)* of nationality: Italy

State *(that is, country)* of residence: Italy

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

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State *(that is, country)* of residence: Italy

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

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- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: Italy

State *(that is, country)* of residence: Italy

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

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- ☐ inventor only *(If this check-box is marked, do not fill in below.)*

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Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

☒ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierra Leone, SZ Swaziland, TZ United Republic of Tanzania, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

☒ AE United Arab Emirates
☒ AL Albania
☒ AM Armenia
☒ AT Austria
☒ AU Australia
☒ AZ Azerbaijan
☒ BA Bosnia and Herzegovina
☒ BB Barbados
☒ BG Bulgaria
☒ BR Brazil
☒ BY Belarus
☒ CA Canada
☒ CH and LI Switzerland and Liechtenstein
☒ CN China
☒ CR Costa Rica
☒ CU Cuba
☒ CZ Czech Republic
☒ DE Germany
☒ DK Denmark
☒ DM Dominica
☒ EE Estonia
☒ ES Spain
☒ FI Finland
☒ GB United Kingdom
☒ GD Grenada
☒ GE Georgia
☒ GH Ghana
☒ GM Gambia
☒ HR Croatia
☒ HU Hungary
☒ ID Indonesia
☒ IL Israel
☒ IN India
☒ IS Iceland
☒ JP Japan
☒ KE Kenya
☒ KG Kyrgyzstan
☒ KP Democratic People's Republic of Korea
☒ KR Republic of Korea
☒ KZ Kazakhstan
☒ LC Saint Lucia
☒ LK Sri Lanka
☒ LR Liberia
☒ LS Lesotho
☒ LT Lithuania
☒ LU Luxembourg
☒ LV Latvia
☒ MA Morocco
☒ MD Republic of Moldova
☒ MG Madagascar
☒ MK The former Yugoslav Republic of Macedonia
☒ MN Mongolia
☒ MW Malawi
☒ MX Mexico
☒ NO Norway
☒ NZ New Zealand
☒ PL Poland
☒ PT Portugal
☒ RO Romania
☒ RU Russian Federation
☒ SD Sudan
☒ SE Sweden
☒ SG Singapore
☒ SI Slovenia
☒ SK Slovakia
☒ SL Sierra Leone
☒ TJ Tajikistan
☒ TM Turkmenistan
☒ TR Turkey
☒ TT Trinidad and Tobago
☒ TZ United Republic of Tanzania
☒ UA Ukraine
☒ UG Uganda
☒ US United States of America
☒ UZ Uzbekistan
☒ VN Viet Nam
☒ YU Yugoslavia
☒ ZA South Africa
☒ ZW Zimbabwe

Check-boxes reserved for designating States which have become party to the PCT after issuance of this sheet:

☒ DZ Algeria
☒ AG Antigua and Barbuda

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except any designation(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation (including fees) must reach the receiving Office within the 15-month time limit.)*

Box No. VI PRIORITY CLAIM ☐ Further priority claim indicated in the Supplemental Box.

Filing date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	regional application:* regional Office	international application: receiving Office
item (1) 09 Apr 1999 (09.04.99)	MI99A 000728	Italy		
item (2)				
item (3)				

☐ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): _____

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(if two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA /

Request to use results of earlier search; reference to that search *(if an earlier search has been carried out by or requested from the International Searching Authority):*

Date *(day/month/year)* Number Country *(or regional Office)*

Box No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	: 04
description (excluding sequence listing part)	: 13
claims	: 01
abstract	: 01
drawings	: --
sequence listing part of description	:
Total number of sheets	: 19

This international application is **accompanied by** the item(s) marked below:

1. ☐ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) identified in Box No. VI as item(s):
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganism or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other *(specify)*: Request for fax acknowledgement

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: English

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).

F Minoja

Fabrizio MINOJA

7 April 2000 (07.04.00)

For receiving Office use only

1. Date of actual receipt of the purported international application: 07 APR 2000 (07.04.2000)
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority (if two or more are competent): ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid.

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
SCB540PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 03100	07/04/2000	09/04/1999
Applicant		
ANTIBIOTICOS S.P.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]P 00/03100

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/385 A61P35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, MEDLINE, WPI Data, PAJ, EMBASE, BIOSIS, CANCERLIT, AIDSLINE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLACCI A ET AL: "Inhibition of chemically induced cell transformation by lipoic acid (Meeting abstract)." PROC ANNU MEET AM ASSOC CANCER RES, (1997). VOL. 38, PP. A2419. ISSN: 0197-016X., XP000929597 Istituto Nazionale per la Ricerca sul Cancro (IST) Biotechnology Satellit Unit, Bologna, Italy 40126. the whole document --- -/--	1,4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2000	31/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cielen, E

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SILINGARDI P; NOONAN D; HORN W; VACCARI M; ARGNANI A; GRILLI S; IACONDINI A; COLACCI A : "Effect of lipoic acid on foci forming capacities of transformed cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 39, March 1998 (1998-03), pages 289-290, XP000929579 cited in the application the whole document	1
X	US 5 679 697 A (GARNETT MERRILL) 21 October 1997 (1997-10-21) abstract column 1, line 10 - line 18 column 4, line 43 - line 65 column 5, line 14 - line 40 column 12, line 33 - line 41 column 13, line 58 -column 14, line 25 column 14, line 65 -column 15, line 19 column 15, line 59 -column 16, line 3 claims	1-4
X	WO 99 06040 A (BERRY CHRISTOPHER J ;PACKER LESTER (US); FOLEY JOHN L (US)) 11 February 1999 (1999-02-11) abstract page 1, line 5 - line 9 page 10, line 13 - line 23 page 13, line 12 - line 16 page 15, line 24 - line 28 page 16, line 26 -page 17, line 2 page 17, line 25 -page 18, line 4 page 19, line 9 - line 14 page 30, line 7 - line 13 claim 1	1,2,4
X	WO 95 13061 A (IMMUNAL KFT ;KULCSAR GYULA (HU)) 18 May 1995 (1995-05-18) abstract page 3, line 13 - line 21 page 4, line 10 - line 20 page 5, line 1 - line 29 page 6, line 1 - line 5 page 16 page 22, line 1 - line 5 claims 1,4,7,9,16,18-30	1-4

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INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]EP 00/03100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 36773 A (UNIV YALE) 27 August 1998 (1998-08-27) abstract page 3, line 16 -page 4, line 3 page 4, line 19 - line 27 page 5, line 6 - line 9 page 13, line 1 - line 9 page 15, line 19 -page 16, line 6 page 23, line 24 - line 28 page 25, line 20 - line 23 table 1 claims ---	1-4
X	CH 683 920 A (MARIGEN SA) 15 June 1994 (1994-06-15) cited in the application abstract page 2, line 1 - line 30 page 4, line 60 -page 5, line 8 page 5, line 41 - line 44 page 15, line 54 - line 56 page 16, line 13 - line 27 page 21, line 30 - line 65 claims ---	1,2,4
E	WO 00 24734 A (UNIV NEW YORK) 4 May 2000 (2000-05-04) abstract page 1, line 10 - line 16 page 5, line 1 - line 25 page 6, line 19 - line 35 page 15, line 9 - line 32 page 17, line 29 -page 18, line 2 page 19, line 5 - line 20 -----	1-4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
P[redacted]EP 00/03100

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5679697 A	21-10-1997	US 5463093 A	31-10-1995
		AU 1180795 A	13-06-1995
		CA 2176603 A	01-06-1995
		EP 0730449 A	11-09-1996
		WO 9514466 A	01-06-1995
		US 5776973 A	07-07-1998
WO 9906040 A	11-02-1999	AU 8768098 A	22-02-1999
WO 9513061 A	18-05-1995	HU 213677 B	29-12-1997
		AU 682735 B	16-10-1997
		AU 1074995 A	29-05-1995
		CA 2151826 A	18-05-1995
		CH 686867 A	31-07-1996
		CN 1116406 A	07-02-1996
		CZ 9501773 A	17-01-1996
		DE 4498692 T	22-02-1996
		EP 0679081 A	02-11-1995
		ES 2094702 A	16-01-1997
		FI 953369 A	07-07-1995
		JP 8508045 T	27-08-1996
		NL 9420013 T	02-10-1995
		PL 309600 A	30-10-1995
		RU 2138257 C	27-09-1999
		SE 9502474 A	06-07-1995
WO 9836773 A	27-08-1998	AU 6436998 A	09-09-1998
CH 683920 A	15-06-1994	NONE	
WO 0024734 A	04-05-2000	AU 1324600 A	15-05-2000

PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

To:
BIANCHETTI BRACCO MINOJA S.r.l.
Attn. Minoja, Fabrizio
Via Rossini, 8
20122 Milano
ITALY

RICEVUTO IL
RECEIVED ON
31 AGO. 2000
BIANCHETTI-BRACCO-MINOJA srl

Applicant's or agent's file reference SCB540PCT	Date of mailing *(day/month/year)* 31/08/2000 **FOR FURTHER ACTION** See paragraphs 1 and 4 below
International application No. PCT/EP 00/ 03100	International filing date *(day/month/year)* 07/04/2000
Applicant ANTIBIOTICOS S.P.A.	

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Mike Iverstam

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When? Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How? Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

MINOJA, Fabrizio et al.
BIANCHETTI BRACCO MINOJA S.r.l.
Via Rossini, 8
20122 Milano
ITALIE

RICEVUTO IL
RECEIVED ON
25 LUG. 2001
BIANCHETTI-BRACCO-MINOJA srl

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	23.07.2001
Applicant's or agent's file reference: SCB540PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/03100	07/04/2000	09/04/1999

Applicant
ANTIBIOTICOS S.P.A. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Almalé Murillo, J-A Tel.+49 89 2399-8059

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
SCB540PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/03100	07/04/2000	09/04/1999
International Patent Classification (IPC) or national classification and IPC A61K31/385		
Applicant ANTIBIOTICOS S.P.A. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
03/11/2000	23.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Hornich, E Telephone No. +49 89 2399 8721

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/03100

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-13 as originally filed

Claims, No.:

1-4 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-4
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-4
Industrial applicability (IA)	Yes:	Claims	1-4 (see separate sheet item 1.)
	No:	Claims	

2. Citations and explanations
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

SECTION V

1. For the assessment of the present claims 1-4 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

2. Reference is made to the following documents:

D1: COLACCI A ET AL: 'Inhibition of chemically induced cell transformation by lipoic acid (Meeting abstract).' PROC ANNU MEET AM ASSOC CANCER RES, (1997). VOL. 38, PP. A2419. ISSN: 0197-016X., Istituto Nazionale per la Ricerca sul Cancro (IST) Biotechnology Satellit Unit, Bologna, Italy 40126.

D2: SILINGARDI P; NOONAN D; HORN W; VACCARI M; ARGNANI A; GRILLI S; IACONDINI A; COLACCI A : 'Effect of lipoic acid on foci forming capacities of transformed cells.' PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 39, March 1998 (1998-03), pages 289-290, cited in the application

D3: US-A-5 679 697

D4: WO 99 06040 A

D5: WO 95 13061 A

D6: WO 98 36773 A

D7: CH 683 920 A

D8: WO 00 24734 A

2.1 Documents **D1** and **D2** identify α-lipoic acid as chemoprotective agent as it strongly reduces the invasive behaviour of 1,2-dibromoethane-transformed BALB/c 3T3 cells (through a reconstituted basal membrane Matrigel^{R}, dose-related).

2.2 Document **D3** provides 'a novel polynucleotide reductase which is a complex

comprising palladium or a salt thereof and lipoic acid or a derivative thereof', useful for the treatment of tumours and metastases and administered through e.g. the oral, intravenous or subcutaneous routes (col. 4, l. 54-59; col. 14, l. 20-23; col. 15, l. 59-66; claims 1, 5, 7, 8, 9).

2.3 **D4** discloses the use of 'a combination of a tocotrienol and α-lipoic acid or a tocotrienyl lipoate or derivatives thereof for the treatment of, among others, cancer or cancer metastases' (p. 10, l. 13-23; p. 12, l. 7-11; p. 15, l. 28; p. 17, l. 25-28; p. 30, l. 7-8; claims 1, 6, 29).

2.4 **D5** relates to a pharmaceutical composition comprising α-lipoic acid which can be, orally or parenterally, applied without toxic effects for prevention of cancerous disease and for hindering metastases formation (see abstract; p. 21, l. 35-38; claims 1, 4, 7, 9, 16, 18, 20, 24, 28, 29).

2.5 Document **D6** involves the use of α-lipoic acid (or salts thereof) for the treatment of (pre)cancer, since it induces apoptosis of cells of (pre)cancer (see abstract; p. 5, l. 6-9; p. 15, l. 24 - p. 16, l. 6; p. 29, table 1; claims 1, 4, 5, 12, 13).

2.6 **D7** describes the effect of esters of α-lipoic acid on the formation of tumours when administered orally, intravenously or subcutaneously (p. 2, l. 3-12; p. 2, l. 20 - p. 4, l. 30; p. 4, l. 60 - p. 5, l. 8; p. 16, l. 13-17; claims 1, 2).

2.7 **D8** describes lipoic acid derivatives used for the prevention and the treatment of primary or metastatic cancers (see abstract; p. 5, l. 3 - p. 6, l. 5; p.6, l. 20-34; p. 17, l. 29 - p. 18, l. 2; p. 19, l. 5-20; claims).

3. *Novelty* **(Art. 33(2) PCT)**

According to the above-cited documents **D1** - **D8**, in particularly **D1** - **D5** and **D8**, the effect of α-lipoic acid or derivatives thereof on cancer metastases is already known. Within **D1** and **D2**, the reduction of the invasive behaviour of cancer cells after treatment with α-lipoic acid is emphasized.

Therefore, novelty of the subject-matter of claims 1 - 4 of the present application **cannot be acknowledged**.

SECTION VIII

4. Formulations as in claim 1, 'use of ... for the preparation of antimetastatic medicaments', are considered by this Authority as claims for the first medical use of an active compound, since the intended use ('antimetastatic medicament') should be disregarded within the examination of a European Patent Application.
However, in interpreting claims for determining novelty, the subject-matter of claim 1 is regarded as 'the use of alpha lipoic acid ... for the preparation of a medicament for the treatment (of the prevention) of metastasis', corresponding to the probable intention of the Applicant (second medical use).

   The same applies to claims 2 - 4 due their dependency on claim 1.

5. It is not evident to which group of compounds the wording 'physiologically equivalent derivatives' in claim 1 refers. Thus, claim 1 and the dependent claim 4 lack clarity in the sense of **Art. 6 PCT**.

6. The use of the proper name 'Matrigel^{R}' (p. 4, l. 33; p. 5, l. 2 and 9) without any further description of the composition, allowing the sufficient identification of the article, is undesirable since it is not clear if the name relates to a range of different products (see the PCT-Guidelines, C-II, 4.16.).

7. The abbreviations 'NCS D-MEM' and 'D-MEM' used in the description of the assay on p. 5, l. 21 and p. 7, l. 4/5 are unclear.

PCT

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(54) Title: THE USE OF ALPHA LIPOIC ACID IN THE ANTIMETASTATIC TREATMENT

(57) Abstract

The invention relates to the use of alpha lipoic acid, also known as lipoic acid, thioctic acid or 1,2–dithiolan–3–pentanoic acid,as well as derivatives thereof, in the control of tumour progression and in the antimetastatic therapy.

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THE USE OF ALPHA LIPOIC ACID IN THE ANTIMETASTATIC TREATMENT

The invention relates to the use of alpha lipoic acid, also known as lipoic acid, thioctic acid or 1,2-dithiolan-3-pentanoic acid, as well as derivatives thereof, in the control of tumour progression and in the antimetastatic therapy.

TECHNOLOGICAL BACKGROUND

It is universally accepted that cancerogenesis is a multiphase process in which at least three development phases are recognised: initiation, promotion and progression (Rous and Kidd, J. Exp. Med., 73: 365-376, 1941; Beremblum and Shubik, Br. J. Cancer 1: 383-386, 1947; Foulds L., Cancer Res., 14: 327-339, 1954). In the progression phase, a cell population is selected which lacks control of proliferation and acquires malignant characteristics, giving rise to the metastatic process. The diagnosis and treatment of tumours usually begin at a late stage when most patients already have occult or overt metastasis. In particular the critical pathological turning point is the initiation of local invasion leading to the dissemination of tumour cells. An important window of therapeutical intervention can be defined as the period during which transition from hyperproliferative state to the acquisition of the capacity for invasion and metastasis occurs (Kohn and Liotta, Cancer Res., 55: 1856-1862, 1995). Treatment with an antimetastatic agent can delay or block the processes of invasion and metastasis, increasing the chance of survival. These drugs should be administered daily and for long-term therapies.

Most recently identified antimetastatic and/or tumour progression inhibiting agents recently found (BB2516 Marimastat, BB94 Batimastat, BB3644 (British Biotech),

determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

In general, the dosage range for the S-antioxidants of the invention will range from about 10 μM to about 50 mM.

5.2. Uses of the S-Antioxidant Compounds and Compositions

The S-antioxidant compounds that are determined to selectively induce apoptosis, and pharmaceutical compositions thereof, can be used for a variety of purposes, as described herein.

The safe and effective amount of the S-antioxidant compound or composition will vary with the particular condition being treated, the age and physical condition of the patient being treated, the severity of the condition, the nature of concurrent treatment, the specific compound, compounds or composition employed, the particular pharmaceutically-acceptable carrier utilized, and like factors within the knowledge, and expertise of the attending physician or health care provider. The teaching provided in Section 5.2.1.2, above, however, can successfully be utilized as a guide to routinely determining useful S-antioxidant dosage ranges.

In particular, the methods of the present invention are useful, first, for selectively inducing apoptosis of precancer cells by administering a safe and effective amount of an S-antioxidant to a subject. Administration results in a reduction in the number of precancer cells present in the subject. An effective dose here refers to that amount of the S-antioxidant compound sufficient to result in selective apoptosis of precancer cells. In a preferred embodiment, the S-antioxidant is topically administered. The precancer cells in which apoptosis can selectively be induced include, but are not limited to cells of the type described in Section 5.2.1., below.

The methods of the present invention are also useful for selectively inducing apoptosis of cancer cells by administering a safe and effective amount of an S-antioxidant to a subject. Administration results in a reduction in the number of cancer cells present in the subject. An effective dose here refers to that amount of the S-antioxidant compound sufficient to result in selective apoptosis of cancer cells and, preferably, a regression of precancer or cancer lesions. In a preferred embodiment, the S-antioxidant is topically administered. The cancer cells which in which apoptosis can selectively be induced include, but are not limited to cells of the type and/or disorders described in Section 5.2.2., below.

The methods of the present invention are also useful for reducing the number of cancer cells present in a subject by administering an S-antioxidant to the subject as an adjunct to chemotherapy or radiation therapies such that the susceptibility of the cancer cells to apoptosis is enhanced relative to the non-cancer cells of the subject. S-antioxidant administration can be performed on a subject undergoing or has undergone chemotherapeutic or radiotherapeutic therapies. The time frame between treatment will vary according to the individual. The cancer cells which in which apoptosis can selectively be induced include, but are not limited to cells of the type and/or disorders described in Section 5.2.2., below.

These methods of the present invention are also useful as an adjuncts to p53 therapy, including p53 gene therapy. S-antioxidant administration can be performed on a subject undergoing or has undergone p53 gene therapy. Such an adjunct to p53 therapy can include, first, administration of pharmaceutical S-antioxidant compositions as described in Section 5.1.2., above, which further comprise a functional p53 polypeptide. In one embodiment, the p53 polypeptide comprises a full length, wild-type human p53 polypeptide. In another embodiment, the p53 polypeptide comprises a portion of a human p53 polypeptide which exhibits p53 function.

Methods for using the S-antioxidant compositions of the invention as adjuncts to p53 gene therapy comprise administration of the S-antioxidant compositions of the invention to a subject undergoing or having undergone p53 gene therapy. Methods for p53 gene therapy are well known to those of skill in the art and can include, for example, WO 97/10007; U.S. Patent No. 5,573,925; and WO 95/11301, which are hereby incorporated by reference in their entirety. Further, for general reviews of the methods of gene therapy, see Goldspiel et al., 1993, Clinical Pharmacy 12:488-505; Wu and Wu, 1991, Biotherapy 3:87-95; Tolstoshev, 1993, Ann. Rev. Pharmacol. Toxicol. 32:573-596; Mulligan, 1993, Science 260:926-932; and Morgan and Anderson, 1993, Ann. Rev. Biochem. 62:191-217; May, 1993, TIBTECH 11(5):155-215). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), 1993, Current Protocols in Molecular Biology, John Wiley & Sons, NY; and Kriegler, 1990, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY.

Still further, the methods of the present invention can be practiced as set forth herein to reduce or inhibit tumor vascularization, to induce differentiation in cancer cells, or to inhibit HIV-1 replication.

In another embodiment of the invention, an S-antioxidant compound of the invention can be administered to treat hyperproliferative or benign dysproliferative disorders. Specific embodiments are directed to treatment or prevention of cirrhosis of the liver (a condition in which scarring has overtaken normal liver regeneration processes), treatment of keloid (hypertrophic scar) formation (disfiguring of the skin in which the scarring process interferes with normal renewal), psoriasis (a common skin condition characterized by excessive proliferation of the skin and delay in proper cell fate determination), benign tumors, fibrocystic conditions, tissue hypertrophy (*e.g.*, prostatic hyperplasia), atherosclerosis, a proliferation of smooth muscle cells

lining blood vessels, restenosis, neointimal hyperplasia and mesangial proliferative nephritis.

5.2.1. Precancer/Premalignant Conditions

The precancer cells in which apoptosis is induced are generally ones which exhibit at least one functional p53 allele. "Functional" as used herein, refers to an ability of the p53 allele to contribute to differential apoptosis in cells. It is to be noted that in certain instances, administration of the S-antioxidant results in restoration of mutant p53 protein conformation and/or activity to normal. Thus, while precancer cells exhibiting at least one functional p53 allele are preferred targets of the methods of the invention, the methods described herein are not to be limited to such cells.

Precancer cells include, but are not limited to cells which present in conditions known or suspected to precede progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 68-79.)

Hyperplasia is a form of controlled cell proliferation involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. As but one example, endometrial hyperplasia often precedes endometrial cancer.

Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplasia can occur in epithelial or connective tissue cells. Atypical metaplasia involves a somewhat disorderly metaplastic epithelium. As but one example, the esophageal metaplasia of Barrett's esophagus often precedes esophageal cancer.

Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form

of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells.

Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation, and is often found in the skin, cervix, respiratory passages, oral cavity, and gall bladder.

Alternatively or in addition to the presence of abnormal cell growth characterized as hyperplasia, metaplasia, or dysplasia, the presence of one or more characteristics of a transformed phenotype, or of a malignant phenotype, displayed *in vivo* or displayed *in vitro* by a cell sample from a patient, can indicate the desirability of therapeutic administration of the S-antioxidant compounds and compositions of the invention.

As mentioned above, such characteristics of a transformed phenotype include morphology changes, looser substratum attachment, loss of contact inhibition, loss of anchorage dependence, protease release, increased sugar transport, decreased serum requirement, expression of fetal antigens, etc. (see also *id.*, at pp. 84-90 for characteristics associated with a transformed or malignant phenotype).

In a specific embodiment, leukoplakia, a benign-appearing hyperplastic or dysplastic lesion of the epithelium, or Bowen's disease, a carcinoma *in situ*, are pre-neoplastic lesions indicative of the desirability of therapeutic intervention.

In another embodiment, fibrocystic disease (cystic hyperplasia, mammary dysplasia, particularly adenosis (benign epithelial hyperplasia)) is indicative of the desirability of therapeutic intervention.

In other embodiments, a patient which exhibits one or more of the following predisposing factors for malignancy is treated by administration of an effective amount of the S-antioxidant compositions of the invention: a chromosomal

translocation associated with a malignancy (*e.g.*, the Philadelphia chromosome for chronic myelogenous leukemia, t(14;18) for follicular lymphoma, etc.), familial polyposis or Gardner's syndrome (possible forerunners of colon cancer), benign monoclonal gammopathy (a possible forerunner of multiple myeloma), and a first degree kinship with persons having a cancer or precancerous disease showing a Mendelian (genetic) inheritance pattern (*e.g.*, familial polyposis of the colon, Gardner's syndrome, hereditary exostosis, polyendocrine adenomatosis, medullary thyroid carcinoma with amyloid production and pheochromocytoma, Peutz-Jeghers syndrome, neurofibromatosis of Von Recklinghausen, retinoblastoma, carotid body tumor, cutaneous melanocarcinoma, intraocular melanocarcinoma, xeroderma pigmentosum, ataxia telangiectasia, Chediak-Higashi syndrome, albinism, Fanconi's aplastic anemia, and Bloom's syndrome; *see* Robbins and Angell, 1976, *Basic Pathology*, 2d Ed., W.B. Saunders Co., Philadelphia, pp. 112-113) etc.)

In another specific embodiment, an S-antioxidant of the invention is administered to a human patient treat an actinic keratinosis condition.

In another specific embodiment, the presence of sun-damaged skin, characterized by lost elasticity, distended capillaries, and individual disordered keratinocytes is indicative of the desirability of therapeutic intervention. Such sun-damaged skin represents a precursor of precancerous actinic keratinosis.

5.2.2. MALIGNANCIES

The cancer cells in which apoptosis is induced are generally ones which exhibit at least one functional p53 allele. "Functional" as used herein, refers to an ability of the p53 allele to contribute apoptosis. It is to be noted that in certain instances, administration of the S-antioxidant results in restoration of mutant p53 protein conformation and/or activity to normal. Thus, while cancer cells exhibiting at least one functional p53 allele are

preferred targets of the methods of the invention, the methods described herein are not to be limited to such cells.

Such cancer cells arise as part of malignancies and related disorders which include but are not limited to those listed in Table 1 (for a review of such disorders, see Fishman et al., 1985, *Medicine*, 2d Ed., J.B. Lippincott Co., Philadelphia):

TABLE 1
MALIGNANCIES AND RELATED DISORDERS

- Leukemia
  - acute leukemia
    - acute lymphocytic leukemia
    - acute myelocytic leukemia
      - myeloblastic
      - promyelocytic
      - myelomonocytic
      - monocytic
      - erythroleukemia
  - chronic leukemia
    - chronic myelocytic (granulocytic) leukemia
    - chronic lymphocytic leukemia
- Polycythemia vera
- Lymphoma
  - Hodgkin's disease
  - non-Hodgkin's disease
- Multiple myeloma
- Waldenström's macroglobulinemia
- Heavy chain disease
- Solid tumors
  - sarcomas and carcinomas
    - fibrosarcoma
    - myxosarcoma
    - liposarcoma
    - chondrosarcoma
    - osteogenic sarcoma
    - chordoma
    - angiosarcoma
    - endotheliosarcoma
    - lymphangiosarcoma
    - lymphangioendotheliosarcoma
    - synovioma
    - mesothelioma
    - Ewing's tumor
    - leiomyosarcoma
    - rhabdomyosarcoma
    - colon carcinoma
    - pancreatic cancer

breast cancer
ovarian cancer
prostate cancer
squamous cell carcinoma
basal cell carcinoma
adenocarcinoma
sweat gland carcinoma
sebaceous gland carcinoma
papillary carcinoma
papillary adenocarcinomas
cystadenocarcinoma
medullary carcinoma
bronchogenic carcinoma
renal cell carcinoma
hepatocellular carcinoma
bile duct carcinoma
choriocarcinoma
seminoma
embryonal carcinoma
Wilms' tumor
cervical cancer
uterine cancer
testicular tumor
lung carcinoma
small cell lung carcinoma
bladder carcinoma
glioma
astrocytoma
medulloblastoma
craniopharyngioma
ependymoma
pinealoma
hemangioblastoma
acoustic neuroma
oligodendroglioma
menangioma
melanoma
neuroblastoma
retinoblastoma

In specific embodiments, malignancy or dysproliferative changes (such as metaplasias and dysplasias), or hyperproliferative disorders, are treated in the bladder, breast, colon, lung, melanoma, pancreas, skin (including, for example, basal cell carcinomas and squamous cell carcinomas) or uterus. In other specific embodiments, sarcoma or leukemia is treated or prevented.

6. EXAMPLE: Antioxidant Action via p53-mediated Apoptosis

The Example presented herein demonstrates that sulfur-containing antioxidants such as N-acetylcysteine (NAC) and dimercaptopropanol (DMP) induced apoptosis in several transformed cell lines and primary cultures, but not in normal cells. In contrast, chain-breaking antioxidants such as vitamin E lacked this activity. An increased glutathione level was not required for apoptosis; however, all apoptosis-inducing antioxidants elevated total cellular thiol levels. Antioxidant-induced apoptosis required the p53 tumor suppressor gene. NAC elevated p53 expression post-transcriptionally, by increasing the rate of p53 mRNA translation rather than by altering protein stability. These observations indicate a redox sensor for p53 induction *in vivo*, with additional transformation-specific information being required for apoptosis.

6.1. Materials And Methods

Cell Lines. MEF primary mouse embryo fibroblast cells (passage <5) and E1A/Ha-ras-transformed MEF were as in Lowe (18). The 308 papilloma cell line, which has a mutant Ha-ras allele (Strickland, J. E. et al., 1988, Cancer Res. 48) and wild-type p53 (Liu, M. et al., 1995, Oncogene 10:1955-1960) were also utilized. The (Rostein, J. B. et al., 1988, Mutation Research 202:421-427)1 mouse embryo fibroblast cell line was as in Levine (Harney, D. M. et al., 1991, Genes Dev. 5:2375-2385). BALB/c 3T3 A31 cells were obtained from the American Type Culture Collection (Bethesda, MD). 308 papilloma cells were maintained in 0.05 mM Ca2+ EMEM medium with 10% fetal bovine serum, and all other cells were grown in DMEM medium with 10% heat-inactivated fetal bovine serum. Cell culture confluence was maintained below 80%.

Chemicals and Cell Treatments. All chemicals were obtained from Sigma, except for Trolox which was purchased from Aldrich. These compounds were freshly dissolved in medium and adjusted to neutral pH if necessary, or (for vitamin E

acetate, Trolox, and BHA) first dissolved in ethanol and added to the medium. Cell viability was determined by trypan blue exclusion as a measure of cell death independent of any growth suppression by p53.

Apoptosis Assays. For fixed cells, apoptosis-associated DNA strand breaks were visualized by fluorescent in situ end-labeling as previously described (Ziegler, A. et al., 1994, Nature 372:773-776). For isolated DNA, DNA fragmentation analysis was performed (Lowe, S. W. et al., 1993, Cell 74:957-967).

For flow cytometry analysis, approximately 10^6 cells per sample were washed with ice-cold PBS and fixed in 95% ethanol. Cells were then resuspended in 1 mg/ml RNase (Sigma) for 30 min at 37 °C and stained with 0.05 mg/ml propidium iodide (Sigma) for 1 h on ice. Flow cytometric analysis was performed with a FACS Vantage flow cytometer (Becton-Dickinson). Cells were excited at 488 nm and the emission was detected through a 630/22 nm band pass filter. A minimum of 10,000 cells were analyzed for each sample. Cell cycle analysis was performed using the Modfit 5.2 software (Verity Software House). Cells were considered to be in apoptosis if they exhibited sub-G1 DNA fluorescence and a forward angle light scatter (FALS) the same as or slightly lower than that of cells in G1 phase (28). Cellular debris was gated out using the electronic threshold.

Northern and Western Blot Analysis. Northern and Western blot analysis were performed as previously described (Liu, M. et al., 1995, Oncogene 10:1955-1960).

Analysis of p53 Protein Synthesis. Cultures of 308 cells were treated in the absence or presence of 20 mM NAC. At 4.5 h post-treatment, cells were incubated with methionine-free medium containing 2% dialyzed and chelexed fetal bovine serum for 0.5 h. At 5 h post-treatment, biosynthetic labeling was initiated by adding 200 μCi of ^{35}S-methionine per ml of

methionine-free medium. The labeling was terminated at 5, 10, or 15 min. Throughout the experiment, 20 mM NAC was included in the group of NAC-treated cells. Cells were then washed twice with 10 ml of ice-cold PBS, scraped, and pelleted by centrifugation at 1500 rpm at 4 °C for 5 min. The supernatant was removed, and the cell pellet was lysed in ice-cold cell lysis buffer (0.5% Triton X-100, 300 mM NaCl, 50 mM Tris-HCl, pH 7.4, 10 μg/ml leupeptin, 0.1 mM phenylmethylsulfonyl fluoride). Aliquots of cell lysate containing equal amounts of protein (30 μg) were subjected to immunoprecipitation analysis with anti-p53 antibody PAb122 (25) and Protein A-agarose (GIBCO-BRL). The immunoprecipitated proteins were resolved on a 10% SDS-PAGE gel. The levels of synthesized p53 protein were then determined by densitometric scanning using a Hewlett-Packard ScanJet 4P Scanner and the NIH image 1.59 analysis software.

Analysis of p53 Protein Half-life. Cultures of 308 cells were treated in the absence or presence of 20 mM NAC. After a 3.5 h incubation, cells were incubated with methionine-free medium containing 2% dialyzed and chelexed fetal bovine serum for 0.5 h. Then cells were labeled by adding 100 μCi of ^{35}S-methionine per ml of methionine-free medium for 1h. At 5 h post-treatment, cells were washed with phosphate-buffered saline and incubated with a chase medium containing a two-fold excess of unlabeled methionine (45 μg/ml) and cysteine (72 μg/ml) for 0, 20, or 40 min. Throughout the experiment, 20 mM NAC was included in the group of NAC-treated cells. Aliquots of each sample lysate were subjected to immunoprecipitation analysis as in the measurement of p53 protein synthesis rate.

Measurement of GSH and Total Thiols. Cells (4 x 106) were harvested from each sample. The GSH-400 kit (R & D Systems) was used following the manufacturer's instructions.

6.2. RESULTS

p53-dependent Apoptosis by N-acetylcysteine. Treatment of murine papilloma line 308 cells with the chemopreventive agent NAC led to dose-dependent cell death (Fig. 1A). Death was apoptotic, with cells showing in situ end-labeling of DNA strand-breaks after 24 h treatment with 20 mM NAC, but not at 6h (data not shown), as well as morphologic changes such as cell shrinkage and nuclear condensation (Fig. 1B). Morphologic changes were minimal in cells treated with doses of NAC associated with high cell viability.

In view of the fact that 308 cells contain a mutant Ha-ras allele and wild-type p53 (Strickland, J. E. et al., 1988, Cancer Res. 48 and Liu, M. et al., 1995, Oncogene 10:1955-19603), a matched pair of normal and transformed cells for comparison was sought. Normal primary MEF cells were compared to a matched line of MEF cells transformed by Ha-ras plus E1A (Lowe, S. W. et al., 1993, Cell 74:957-967). As shown in Fig. 2A-B, the transformed fibroblasts (tMEF $p53^{-/-}$) were sensitive to NAC-induced apoptosis, but their normal counterparts (MEF $p53^{+/+}$) were strikingly resistant. In contrast, both transformed and normal primary cells from $p53^{-/-}$ null mice were deficient in apoptosis induced by NAC (Fig. 2A). A specificity of apoptosis toward transformed cells has been observed previously with chemotherapeutic agents and with hypoxia (Lowe, S. W. et al., 1993, Cell 74:957-967 and Graeber, T. G. et al., 1996, Nature 379:88-91). The specificity of apoptosis toward transformed cells was not due to the level of p53 induction alone, because p53 was induced in their normal counterparts (MEF $p53^{+/+}$) without causing apoptosis (Fig. 2A and 2C). An additional, transformation-related, signal is evidently also required for apoptosis. Apoptosis in response to transformation or other heritable abnormalities has been observed in other systems (Lowe, S. W. et al., 1993, Cell 74:957-967; Graeber, T. G. et al., 1996, Nature 379:88-91; Symonds, H. et al., 1994; Cell 78:703-711; Morgenbesser, S.

D. et al., 1994, Nature 371:72-74; and Brash, D. E., Nature Medicine 2:525-526).

p53 Induction by NAC via Increased p53 Translation Rate.

The molecular mediator of antioxidant-induced apoptosis was next investigated. The tumor suppressor protein p53 is required for induction of apoptosis in response to DNA-damaging agents such as g- or UV-irradiation (Lowe, S. W. et al., 1993, Cell 74:957-967 and Ziegler, A. et al., 1994, Nature 372:773-776), and after hypoxia as well (Graeber, T. G. et al., 1996, Nature 379:88-91). As shown in Fig. 3A, treatment of 308 cells with NAC resulted in a dose-dependent 5- to 10-fold increase of p53 protein levels within 3 to 8 hours. Northern blot analysis revealed no major difference in the steady-state level of p53 mRNA between control and NAC-treated cells (Fig. 3A), indicating that p53 induction was controlled at the post-transcriptional level. NAC also induced p53 in the murine fibroblast cell line BALB/c 3T3 A31.

Of all p53-inducing agents, most damage DNA (Levine, A. J., 1997, Cell *88:* 323-331). Some of these agents increase p53 post-transcriptionally (Kastan, B. K. et al., 1991, Cancer Res. 51:6304-6311; Fritsche, M. et al., 1993, Oncogene 8:307-318 and Liu, M. et al., 1994, Carcinogenesis 15:1089-1092). In some cases, the induction has been shown to be due to the increased p53 protein stability (Fritsche, M. et al., 1993, Oncogene 8:307-318; Liu, M. et al., 1994, Carcinogenesis 15:1089-1092; Maltzman, W. et al., 1984, Mol. Cell. Biol. 4:1689-1694; and Price, B. D. et al., 1993, Oncogen 8:3055-3062). NAC, in contrast, does not induce DNA damage (Yunis, A. A. et al., 1986, Respiration 50(Suppl):50-55; Chan, J. Y. H. et al., 1986, Carcinogenesis 7:1621-1624 and Solen, G., 1993, Int. J. Radiat. Biol. 64:359-366). In order to determine the precise molecular mechanism(s) for the induction of p53 in response to NAC treatment (Fig 3A), both the biosynthetic rate of p53 protein and the p53 protein half-life in 308 cells were directly

measured. As shown in Fig. 3B-C, the biosynthetic rate of p53 protein was elevated by nearly 5-fold after NAC treatment. In contrast, the half-life of p53 protein was not altered in the presence of NAC. These results indicate that enhanced translation of p53 mRNA, and not increased protein stability, accounts for the induction of p53 protein following NAC exposure.

Apoptosis by Other Sulfur-containing Antioxidants.

Because NAC is well-known to ameliorate oxidative stress (Flora, S. D. et al., 1992, Cancer Chemoprevention, pp.; Aruoma, O. I. et al., 1989, Free Rad. Biol. Med. 6:593-597, 1989), the capacity of other antioxidants (Anderson, M. E. et al., 1987, Methods in Enzymology 143:313-325; Ceconi, C. et al., 1990, Cardioscience 1:191-198; and Packer, L. et al., 1995, Free Radical Biology & Medicine) for transformation-specific apoptosis was investigated. As demonstrated in Fig. 4A-4F, the sulfur-containing reducing agents 2,3-dimercaptopropanol (DMP) and L-2-oxo-4-thiazolidinecarboxylate (OTC) also selectively induced apoptosis in E1A/Ha-ras transformed cells, but not in their normal counterparts. Lipoic acid behaved similarly. DMP was active at doses as low as 50 μM. DMP, OTC, and lipoic acid also required p53 (Fig. 4A-4B). These agents, as well as NAC, all induced apoptosis in the human p53+/+ colorectal carcinoma cell line RKO.

In contrast, the nonsulfur-containing antioxidants vitamin E acetate (tocopherol acetate), BHA, and the water-soluble analog of vitamin E, Trolox (Jacobson, M. D. et al., 1995, Nature 374:814-816), had little effect on cell viability of $p53^{+/+}$ tMEF for at least 48 h (Fig. 5). The chosen antioxidant concentrations here were basically the highest soluble or non-cytotoxic doses to $p53^{-/-}$ tMEF. DNA analysis also confirmed that no apoptosis occurred in $p53^{+/+}$ tMEF cells treated with these chain-breaking antioxidants. Thus, the significant feature of these sulfur-containing compounds appears to be their effect on intracellular redox

potential rather than their effect on radical species. In fact, all of the apoptosis-inducing agents tested above elevate cellular thiol levels (Fig. 6).

Glutathione-independence of NAC-induced Apoptosis. A major intracellular pathway of NAC metabolism is deacetylation to the thiol cysteine, the limiting amino acid precursor for synthesis of glutathione (GSH) (Burgunder, J. M. et al., 1989, Eur. J. Clin. Pharmacol.). GSH, in turn, is the major cellular antioxidant (Glutathione: Chemical, Biochemical and Medical Aspects, Vol.). To test the possibility that NAC acts by increasing the level of GSH, we pretreated and co-incubated cells with L-buthionine sulfoximine (BSO); this agent inhibits all GSH synthesis by inactivating g-glutamylcysteine synthetase (Glutathione: Chemical, Biochemical and Medical Aspects, Vol.). As expected, Fig. 6A shows that BSO completely blocks induction of cellular GSH by NAC, while only partially blocking the induction of total thiols (Fig. 6B). However, BSO did not block NAC-induced apoptosis (Fig. 6C), implying that NAC exerts its redox effect directly rather than by increasing GSH.

In this study, it was demonstrated that BSO cannot block NAC-induced apoptosis, although it inhibits cellular GSH elevation by NAC (Fig. 4A-4F). This finding indicates that the present apoptosis differs from the BSO-sensitive biphasic toxicity (Fenton reaction) of some antioxidants other than NAC (Held, K. D. et al., 1996, Radiation Research 145:542-553). Furthermore, it was found that penicillamine (50 μM), a potent chelator of copper, had no effect on NAC-induced apoptosis.

Chain-breaking antioxidants such as vitamin E acetate and Trolox did not induce p53-dependent apoptosis of transformed MEF (Fig. 5). While the p53-dependent mechanism appears to reflect changes in redox potential only, the non-p53 apoptosis pathway appears to involve radical species (Chinery, R. C. et al., 1997, Nature Medicine 3:1233-1241;

and Kastan, M. B., 1997, Nature Medicine 3:1192-1193). A possible pathway relating the two mechanisms is shown in Fig. 7.

The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

Various references are cited herein, the disclosures of which are incorporated by reference in their entireties.

WHAT IS CLAIMED IS:

1. A method for selectively inducing apoptosis of precancer cells in a subject, comprising administering to the subject an amount of a sulphur-containing antioxidant effective to selectively induce apoptosis of precancer cells.

2. The method of Claim 1, wherein the sulphur-containing antioxidant is administered topically.

3. The method of Claim 1, wherein the sulphur-containing antioxidant is administered internally.

4. The method of Claim 3, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

5. The method of Claim 1, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

6. The method of Claim 1 further comprising administering to the subject a composition comprising a purified p53 polypeptide.

7. The method of Claim 1, wherein the subject is undergoing or has undergone p53 gene therapy.

8. The method of Claim 1, wherein the precancer cells are actinic keratinocytes.

9. A method of treating a precancer disorder in a subject in need of such treatment, comprising administering to the subject an amount of a sulphur-containing antioxidant effectively to treat the precancer.

10. The method of Claim 9, wherein the sulphur-containing antioxidant is administered topically.

11. The method of Claim 9, wherein the sulphur-containing antioxidant is administered internally.

12. The method of Claim 11, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

13. The method of Claim 9, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

14. The method of Claim 9 further comprising administering to the subject a composition comprising a purified p53 polypeptide.

15. The method of Claim 9 further comprising administering to the subject an amount of a nucleic acid molecule encoding a p53 polypeptide such that the nucleic acid molecule is expressed in the subject.

16. The method of Claim 9, wherein the subject is undergoing or has undergone p53 gene therapy.

17. The method of Claim 9, wherein the precancer disorder is actinic keratinosis.

18. A method for selectively inducing apoptosis of cancer cells in a subject, comprising administering to the subject an amount of a sulphur-containing antioxidant effective to selectively induce apoptosis of cancer cells.

19. The method of Claim 18, wherein the sulphur-containing antioxidant is administered topically.

20. The method of Claim 18, wherein the sulphur-containing antioxidant is administered internally.

21. The method of Claim 20, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

22. The method of Claim 18, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

23. The method of Claim 18 further comprising administering to the subject a composition comprising a purified p53 polypeptide.

24. The method of Claim 18, wherein the cancer cells are melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, adenocarcinoma cells, sweat gland carcinoma cells, sebaceous gland carcinoma cells, papillary carcinoma cells or papillary adenocarcinoma cells.

25. The method of Claim 18, wherein the subject is undergoing or has undergone a chemotherapeutic treatment for cancer.

26. The method of Claim 25, wherein the sulphur-containing antioxidant is administered topically.

27. The method of Claim 25, wherein the sulphur-containing antioxidant is administered internally.

28. The method of Claim 27, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

29. The method of Claim 25, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

30. The method of Claim 25 further comprising administering to the subject a composition comprising a purified p53 polypeptide.

31. The method of Claim 25, wherein the cancer cells are melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, adenocarcinoma cells, sweat gland carcinoma cells, sebaceous gland carcinoma cells, papillary carcinoma cells or papillary adenocarcinoma cells.

32. The method of Claim 18, wherein the subject is undergoing or has undergone a radiotherapeutic treatment.

33. The method of Claim 32, wherein the sulphur-containing antioxidant is administered topically.

34. The method of Claim 32, wherein the sulphur-containing antioxidant is administered internally.

35. The method of Claim 34, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

36. The method of Claim 32, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

37. The method of Claim 32 further comprising administering to the subject a composition comprising a purified p53 polypeptide.

38. The method of Claim 32, wherein the cancer cells are melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, adenocarcinoma cells, sweat gland carcinoma cells, sebaceous gland carcinoma cells, papillary carcinoma cells or papillary adenocarcinoma cells.

39. The method of Claim 18, wherein the subject is undergoing or has undergone p53 gene therapy.

40. The method of Claim 39, wherein the sulphur-containing antioxidant is administered topically.

41. The method of Claim 39, wherein the sulphur-containing antioxidant is administered internally.

42. The method of Claim 39, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

43. The method of Claim 39, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

44. The method of Claim 39, wherein the cancer cells are melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, adenocarcinoma cells, sweat gland carcinoma cells, sebaceous gland carcinoma cells, papillary carcinoma cells or papillary adenocarcinoma cells.

45. A method of treating cancer, comprising administering to a subject in need of such treatment an amount of a sulphur-containing antioxidant effective to treat the cancer.

46. The method of Claim 45, wherein the sulphur-containing antioxidant is administered topically.

47. The method of Claim 45, wherein the sulphur-containing antioxidant is administered internally.

48. The method of Claim 47, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

49. The method of Claim 45, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

50. The method of Claim 45 further comprising administering to the subject a composition comprising a purified p53 polypeptide.

51. The method of Claim 45 further comprising administering to the subject an amount of a nucleic acid molecule encoding a p53 polypeptide such that the nucleic acid molecule is expressed in the subject.

52. The method of Claim 45, wherein the cancer cells are melanoma cells, basal cell carcinoma cells, squamous cell carcinoma cells, adenocarcinoma cells, sweat gland carcinoma cells, sebaceous gland carcinoma cells, papillary carcinoma cells or papillary adenocarcinoma cells.

53. The method of Claim 45, wherein the subject is undergoing or has undergone a chemotherapeutic treatment for the cancer.

54. The method of Claim 45, wherein the subject is undergoing or has undergone a radiotherapeutic treatment for the cancer.

55. The method of Claim 45, wherein the subject is undergoing or has undergone p53 gene therapy.

56. A method for inhibiting HIV replication comprising administering to a subject infected with HIV an amount of a sulphur-containing antioxidant effective to inhibit HIV replication.

57. The method of Claim 56, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

58. The method of Claim 56 further comprising administering to the subject a composition comprising a purified p53 polypeptide.

59. A method for selectively inducing apoptosis of cells of a hyperproliferative or benign dysproliferative disorder in a subject, comprising administering to the subject an effective amount of a sulphur-containing antioxidant.

60. The method of Claim 59, wherein the sulphur-containing antioxidant is administered topically.

61. The method of Claim 59, wherein the sulphur-containing antioxidant is administered internally.

62. The method of Claim 61, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

63. The method of Claim 59, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

64. The method of Claim 59 further comprising administering to the subject a composition comprising a purified p53 polypeptide.

65. A method of treating a hyperproliferative or benign dysproliferative disorder, comprising administering to a subject in need of such treatment an amount of a sulphur-containing antioxidant effective to treat the disorder.

66. The method of Claim 65, wherein the sulphur-containing antioxidant is administered topically.

67. The method of Claim 65, wherein the sulphur-containing antioxidant is administered internally.

68. The method of Claim 67, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

69. The method of Claim 65, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

70. The method of Claim 65 further comprising administering to the subject a composition comprising a purified p53 polypeptide.

71. A topical formulation comprising a sulphur-containing antioxidant selected from the group consisting of 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid, in a cream, ointment, or lotion..

72. The topical formulation of Claim 71, wherein the topical formulation further comprises a sunscreen.

73. The topical formulation of Claim 71, wherein the topical formulation further comprises a cosmetic.

74. The topical formulation of Claim 71, further comprising a p53 polypeptide.

75. The topical formulation of Claim 71, further comprising a nucleic acid molecule encoding a p53 polypeptide capable of being expressed in a suitable host cell.

76. A method of preventing a precancer, cancer, hyperproliferative or benign dysproliferative disorder in a human subject, comprising administering to the subject an effective amount of a sulphur-containing antioxidant.

77. The method of Claim 76, wherein the sulphur-containing antioxidant is administered topically.

78. The method of Claim 76, wherein the sulphur-containing antioxidant is administered internally.

79. The method of Claim 78, wherein the sulphur-containing antioxidant is administered orally, parenterally or intralesionally.

80. The method of Claim 76, wherein the sulphur-containing antioxidant is selected from the group consisting of N-acetylcysteine, 2,3-dimercaptopropanol, L-2-oxo-4-thiazolidinecarboxylate and lipoic acid.

81. The method of Claim 76 further comprising administering to the subject an effective amount of a p53 polypeptide.

82. A pharmaceutical composition comprising (a) an amount of sulphur-containing antioxidant effective to selectively induce apoptosis in a precancer or cancer cell.

(b) a purified P53 polypeptide or a purified nucleic acid encoding and capable of expressing a p53 polypeptide in a suitable host cell; and (c) a pharmaceutically acceptable carrier.

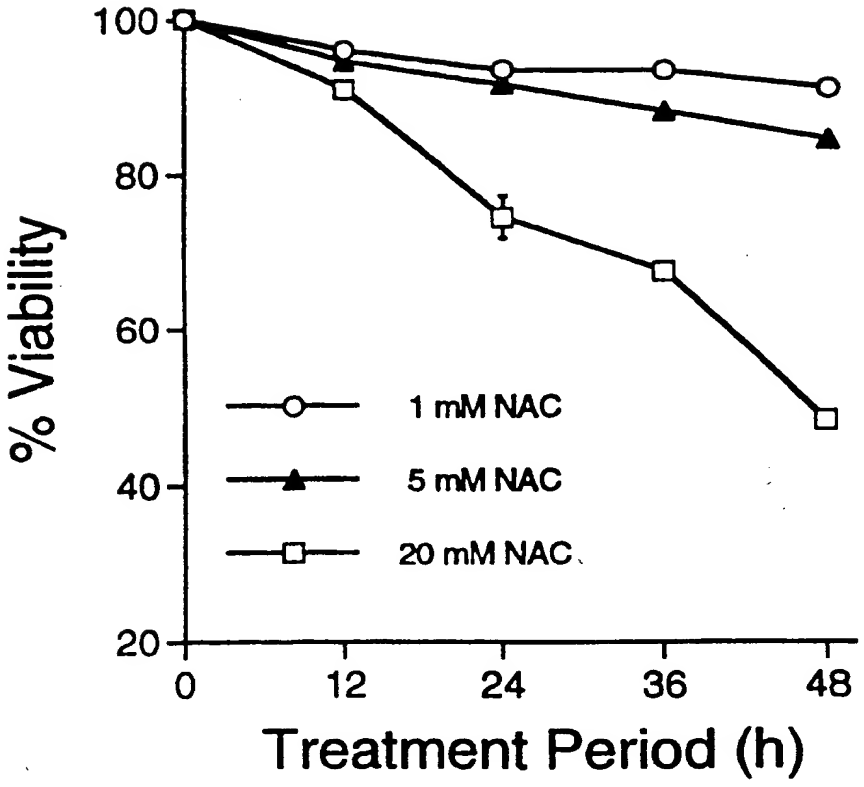

FIG. 1A

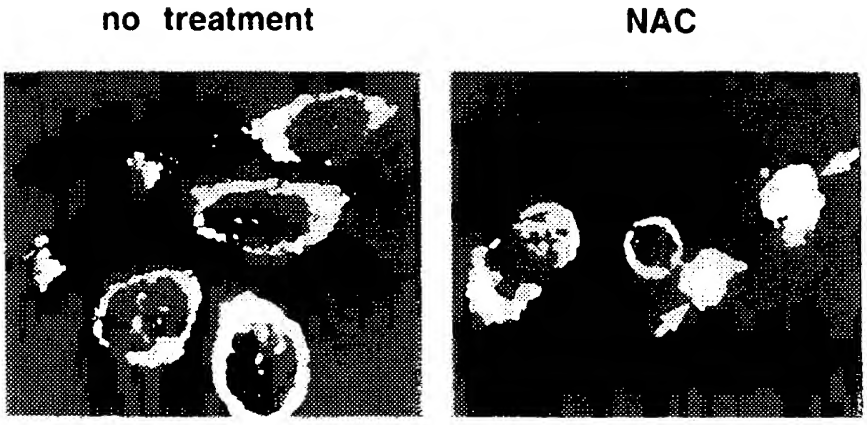

FIG. 1B

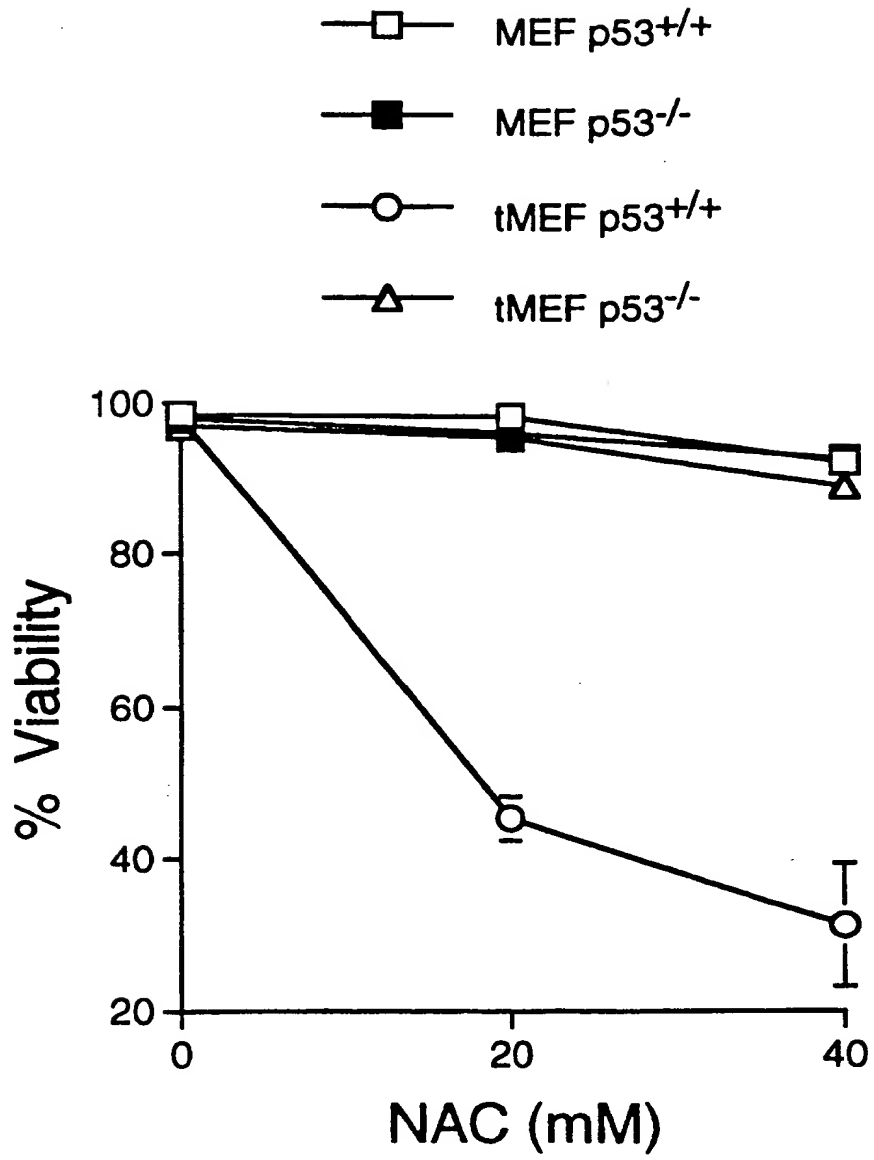

FIG.2A

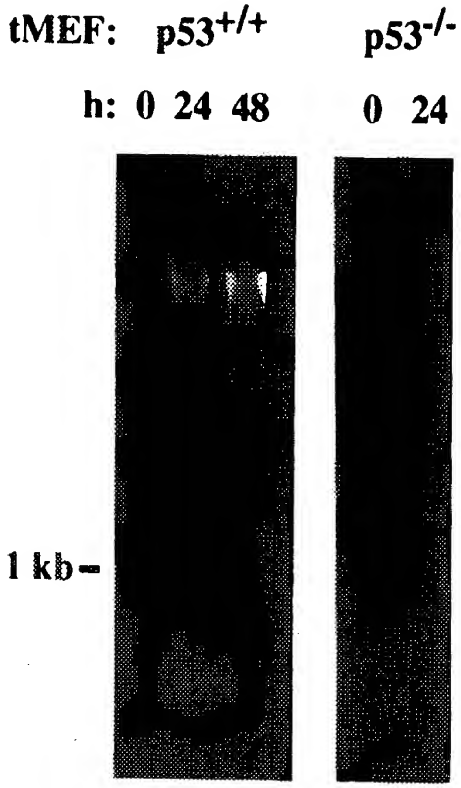

FIG.2B

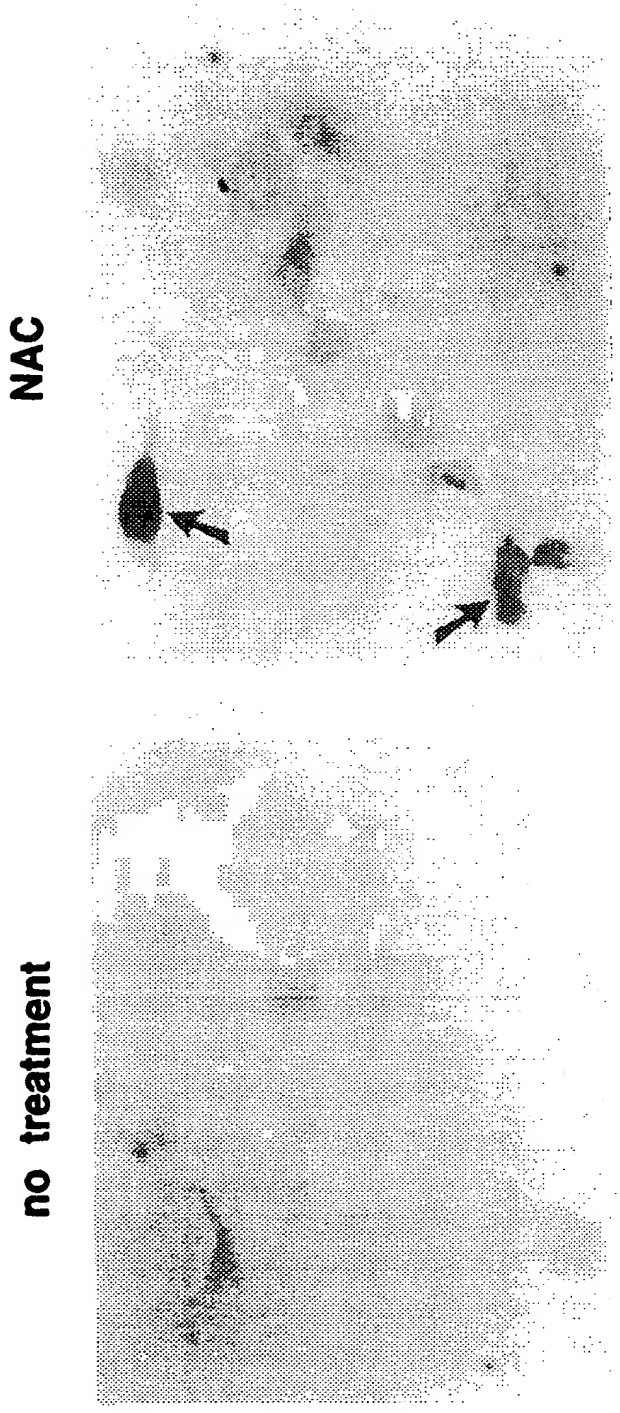

FIG.2C

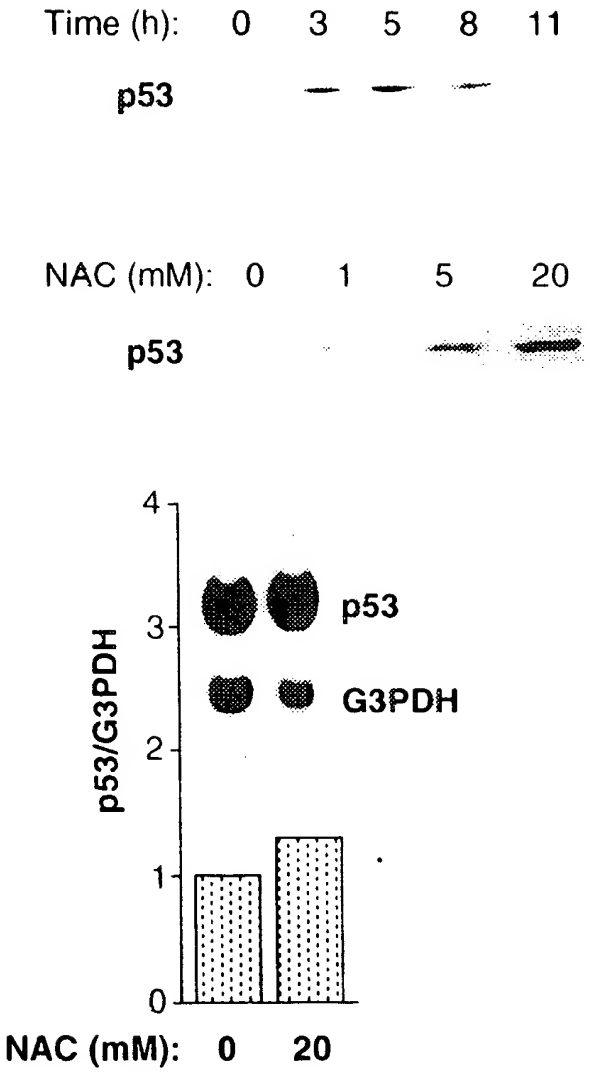

FIG.3A

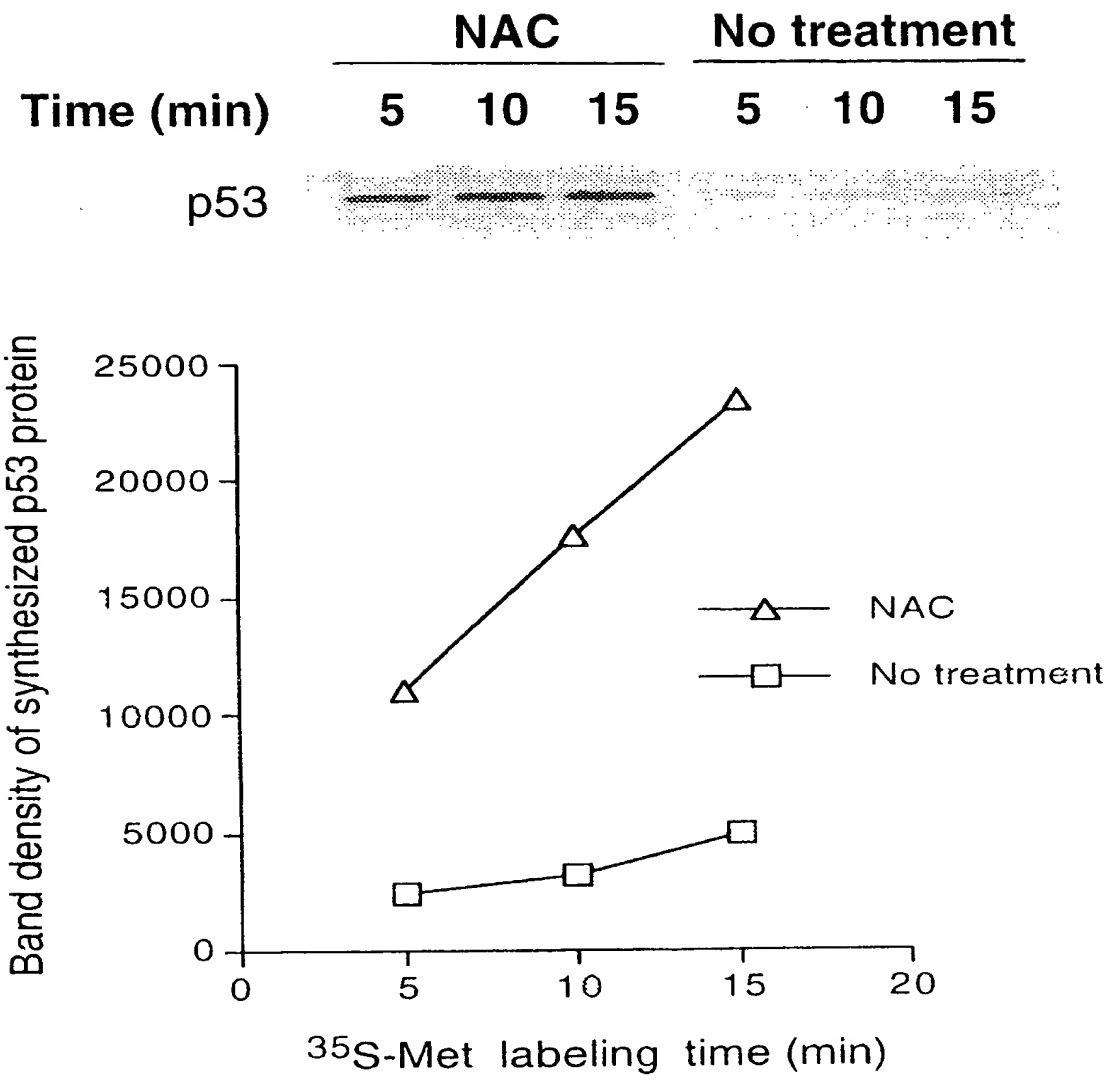

FIG.3B

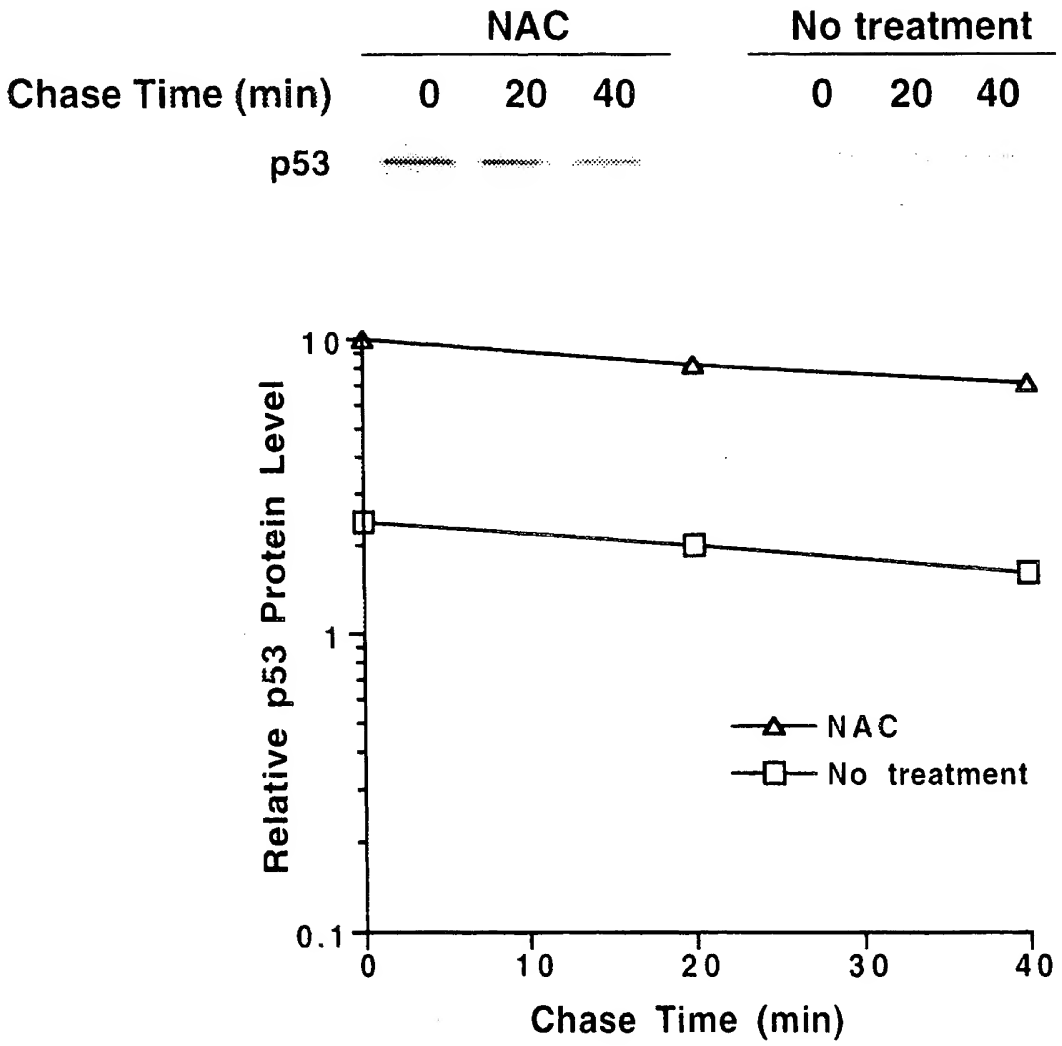

FIG.3C

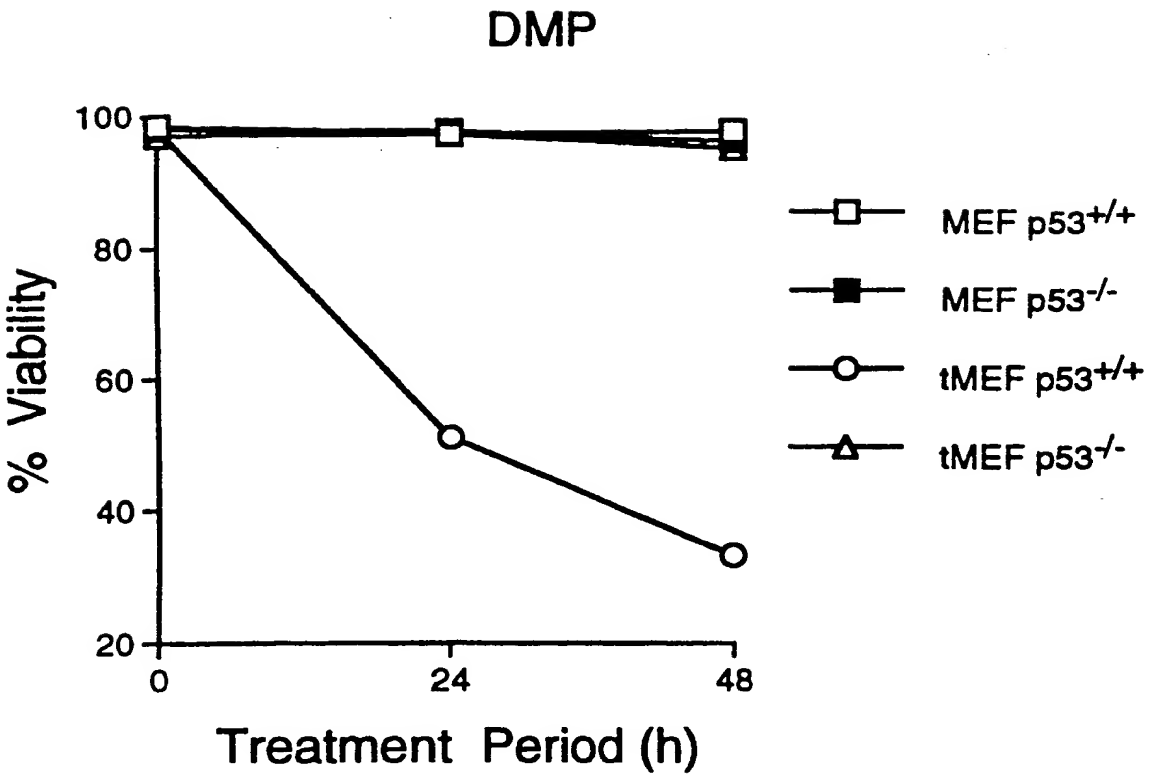

FIG.4A

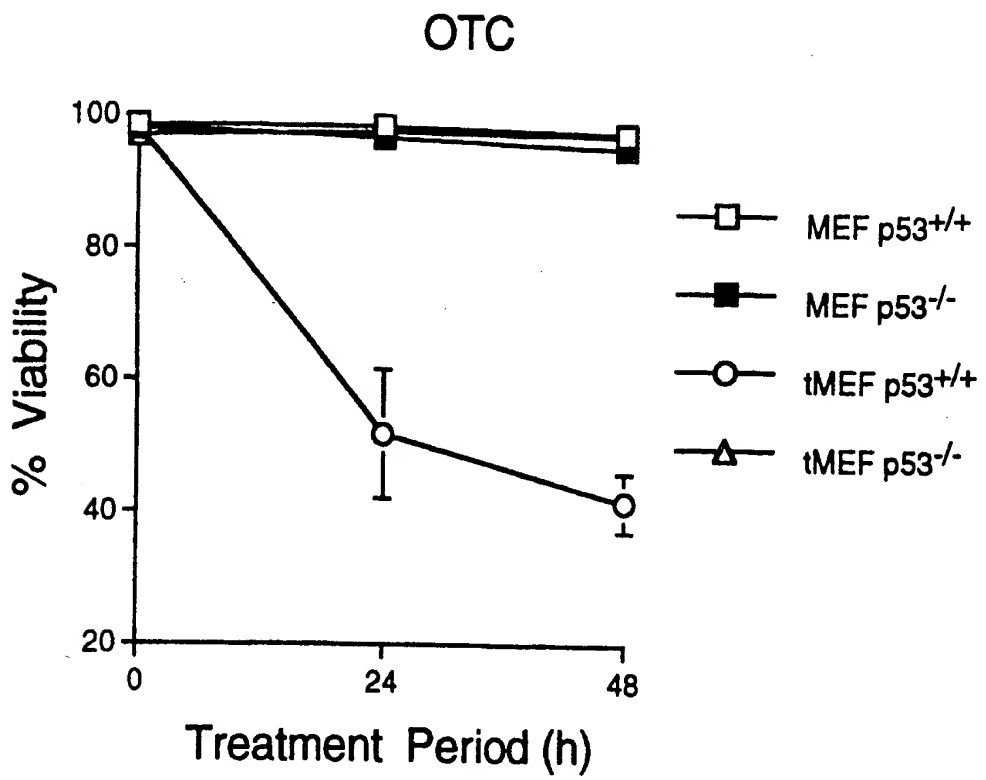

FIG.4B

FIG.4C

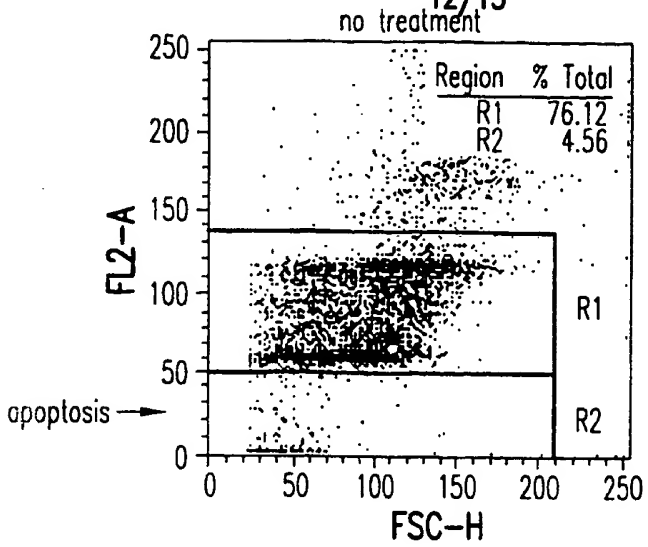

FIG.4D

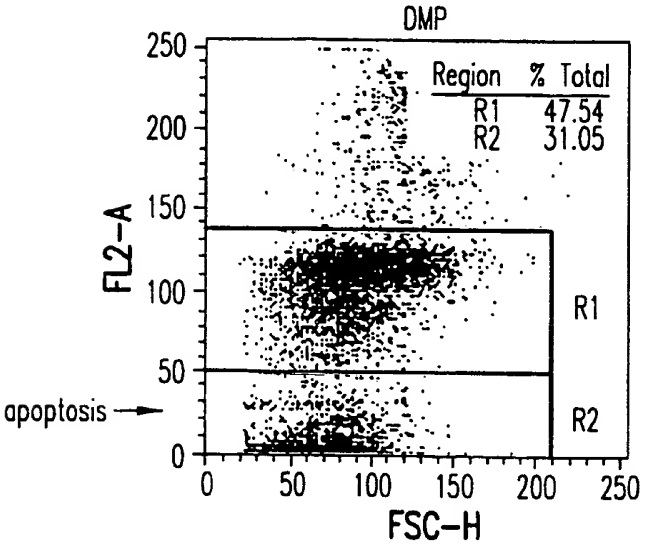

FIG.4E

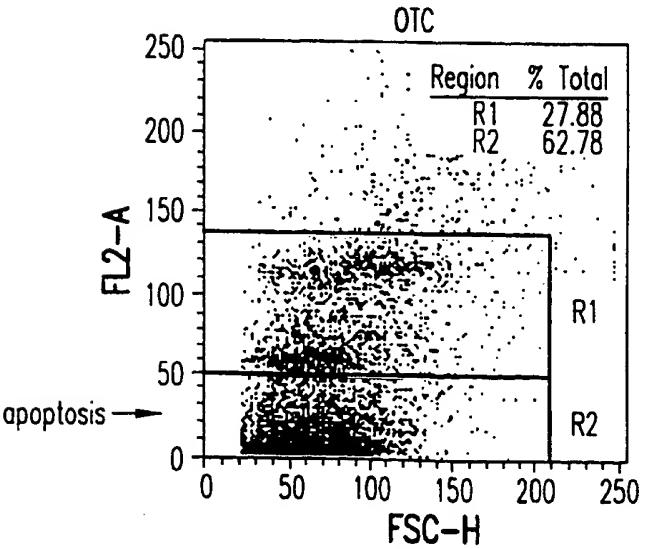

FIG.4F

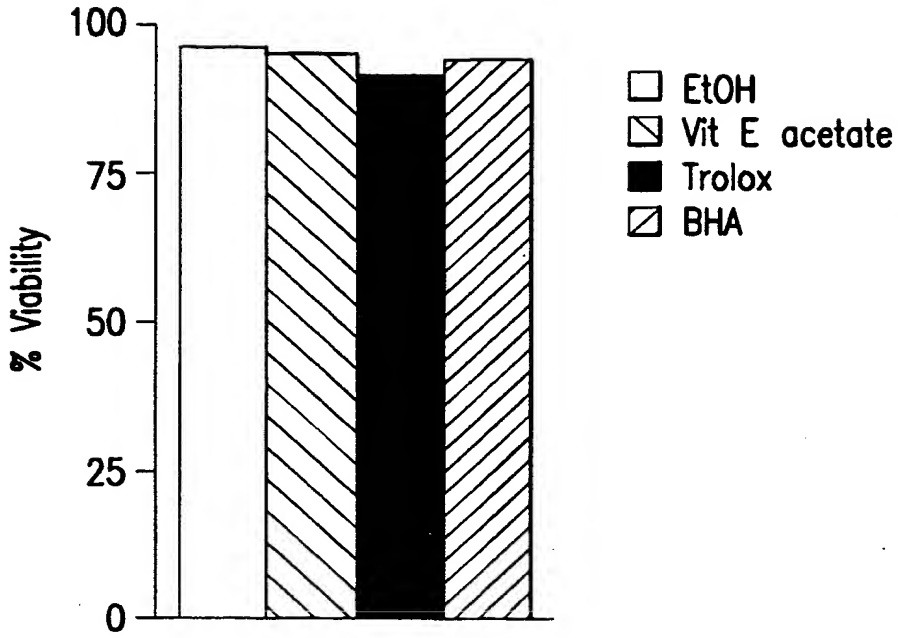

FIG.5

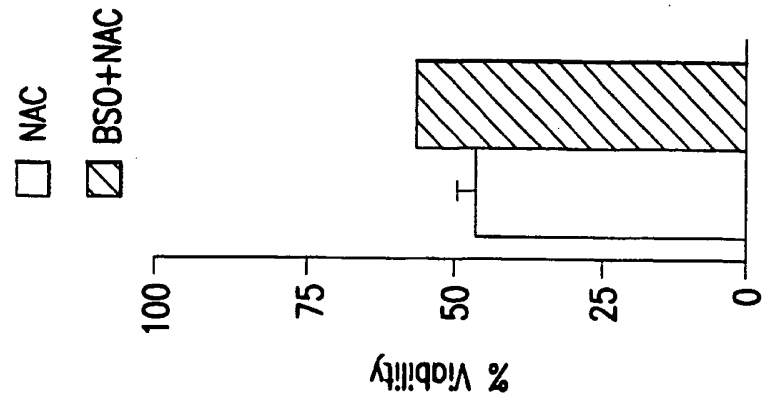

FIG. 6C

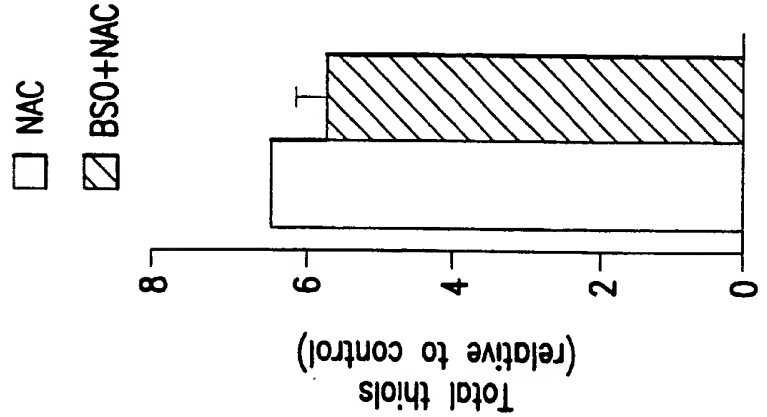

FIG. 6B

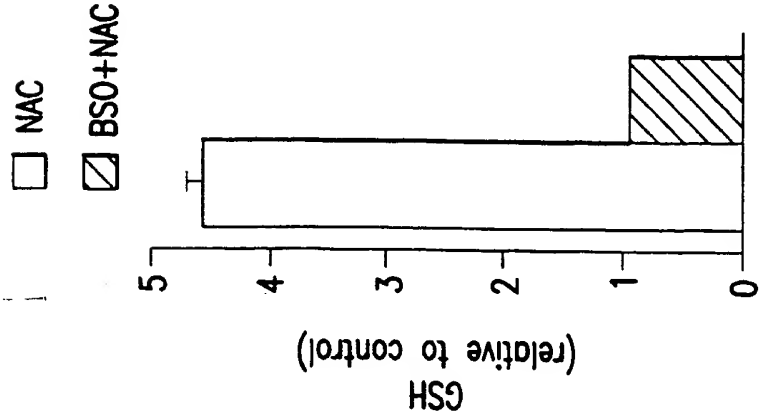

FIG. 6A

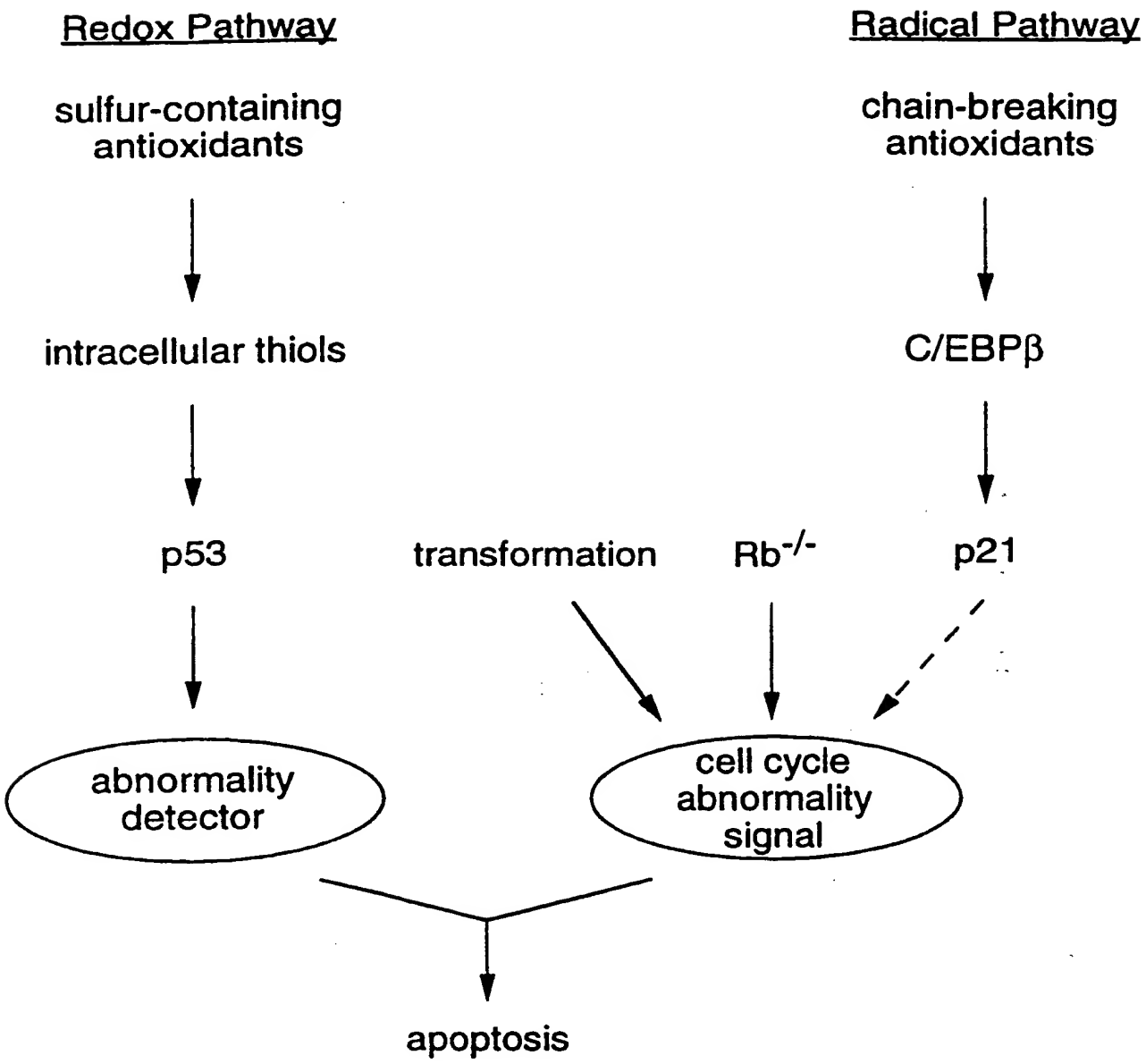

FIG.7

INTERNATIONAL SEARCH REPORT

International application No.: PCT/US98/03296

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 39/39
US CL :530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: MEDLINE,BIOSIS,EMBASE
search terms: p53, acetylcysteine, apoptosis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LOTEM et al. Cellular Oxidative Stress and the Control of Apoptosis by Wild-Type p53, Cytotoxic Compounds and Cytokines Proc. Natl. Acad. Sci. August 1996. Vol 93. pages 9166-9171, especially page 9166-9168 and 9170.	1-82
X	VERHAEGH et al. Redox Regulation of the p53 Tumor Suppressor Protein Proc. Am. Assoc. Canc. Res. March 1996. Vol 37. pages 1-2, entire abstract.	1-82
X	FESUS et al. Probing the Molecular Program of Apoptosis by Cancer Chemopreventive Agents. J. Cell. Biochem. 1995. Vol 58. Supp 22. pages 151-161, especially pages 158-160.	1-82

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search: 06 MAY 1998

Date of mailing of the international search report: 04 JUN 1998

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer: PATRICK B. DELANEY

Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

BAY129566 (Bayer), AG3340 (Agouron), CGS27023A (Novartis), RO32-3555 (Roche), D2163, D5410 (Chiroscience), Metastat (CollaGenex) are synthetic derivatives and have high pharmacological effectiveness. These compounds share an inhibiting activity on metalloproteinases, which participate in degradation of the basal membrane. Unfortunately, therapeutical doses often involve adverse toxic effects (musculo-skeletal, hepatic and gastric toxicities), which prevent long-term treatments as well as repeated daily administrations, although making it possible their use in therapy courses. Furthermore, therapy courses are particularly expensive. Another class of antimetastatic agents is possibly represented by natural polypeptides (TIMPs) (Albini, Pathol. Oncol. Res. 4, 3: 230-241,1998). Obviously, these molecules cannot be administered through the oral route, have low membrane permeability and high costs.

It has been reported that alpha lipoic acid, a known antioxidant molecule used in clinical practice as a therapeutic agent for liver disorders, can be used in different pathologies such as arthritis, ulcer, HIV infection (EP 427287). Alpha lipoic acid is a natural compound, with poor or no adverse effects even at high dosages in humans. Alpha lipoic acid esters were claimed as antineoplastic (CH 683,920) and antitumoral (DE 4400843) agents.

The capability of lipoic acid of inhibiting the malignant transformation of cell lines was described by Colacci et al., and by Silingardi et al., respectively at the 88th Annual Meeting of the American Association for Cancer Research, San Diego, California, USA, April 12-16, 1997, and at the 89th Annual Meeting of the American Association for Cancer Research, New Orleans, Louisiana, USA, March 28- April 1, 1998.

INTERNATIONAL SEARCH REPORT

Ir. tional Application No
PCT/EP 00/03100

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/385 A61P35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, MEDLINE, WPI Data, PAJ, EMBASE, BIOSIS, CANCERLIT, AIDSLINE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLACCI A ET AL: "Inhibition of chemically induced cell transformation by lipoic acid (Meeting abstract)." PROC ANNU MEET AM ASSOC CANCER RES, (1997). VOL. 38, PP. A2419. ISSN: 0197-016X., XP000929597 Istituto Nazionale per la Ricerca sul Cancro (IST) Biotechnology Satellit Unit, Bologna, Italy 40126. the whole document	1,4

-/—

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2000	31/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Cielen, E

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/03100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SILINGARDI P; NOONAN D; HORN W; VACCARI M; ARGNANI A; GRILLI S; IACONDINI A; COLACCI A : "Effect of lipoic acid on foci forming capacities of transformed cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 39, March 1998 (1998-03), pages 289-290, XP000929579 cited in the application the whole document	1
X	US 5 679 697 A (GARNETT MERRILL) 21 October 1997 (1997-10-21) abstract column 1, line 10 - line 18 column 4, line 43 - line 65 column 5, line 14 - line 40 column 12, line 33 - line 41 column 13, line 58 -column 14, line 25 column 14, line 65 -column 15, line 19 column 15, line 59 -column 16, line 3 claims	1-4
X	WO 99 06040 A (BERRY CHRISTOPHER J ;PACKER LESTER (US); FOLEY JOHN L (US)) 11 February 1999 (1999-02-11) abstract page 1, line 5 - line 9 page 10, line 13 - line 23 page 13, line 12 - line 16 page 15, line 24 - line 28 page 16, line 26 -page 17, line 2 page 17, line 25 -page 18, line 4 page 19, line 9 - line 14 page 30, line 7 - line 13 claim 1	1,2,4
X	WO 95 13061 A (IMMUNAL KFT ;KULCSAR GYULA (HU)) 18 May 1995 (1995-05-18) abstract page 3, line 13 - line 21 page 4, line 10 - line 20 page 5, line 1 - line 29 page 6, line 1 - line 5 page 16 page 22, line 1 - line 5 claims 1,4,7,9,16,18-30	1-4

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Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/03100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 36773 A (UNIV YALE) 27 August 1998 (1998-08-27) abstract page 3, line 16 -page 4, line 3 page 4, line 19 - line 27 page 5, line 6 - line 9 page 13, line 1 - line 9 page 15, line 19 -page 16, line 6 page 23, line 24 - line 28 page 25, line 20 - line 23 table 1 claims	1-4
X	CH 683 920 A (MARIGEN SA) 15 June 1994 (1994-06-15) cited in the application abstract page 2, line 1 - line 30 page 4, line 60 -page 5, line 8 page 5, line 41 - line 44 page 15, line 54 - line 56 page 16, line 13 - line 27 page 21, line 30 - line 65 claims	1,2,4
E	WO 00 24734 A (UNIV NEW YORK) 4 May 2000 (2000-05-04) abstract page 1, line 10 - line 16 page 5, line 1 - line 25 page 6, line 19 - line 35 page 15, line 9 - line 32 page 17, line 29 -page 18, line 2 page 19, line 5 - line 20	1-4

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/03100

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5679697 A	21-10-1997	US 5463093 A	31-10-1995
		AU 1180795 A	13-06-1995
		CA 2176603 A	01-06-1995
		EP 0730449 A	11-09-1996
		WO 9514466 A	01-06-1995
		US 5776973 A	07-07-1998
WO 9906040 A	11-02-1999	AU 8768098 A	22-02-1999
WO 9513061 A	18-05-1995	HU 213677 B	29-12-1997
		AU 682735 B	16-10-1997
		AU 1074995 A	29-05-1995
		CA 2151826 A	18-05-1995
		CH 686867 A	31-07-1996
		CN 1116406 A	07-02-1996
		CZ 9501773 A	17-01-1996
		DE 4498692 T	22-02-1996
		EP 0679081 A	02-11-1995
		ES 2094702 A	16-01-1997
		FI 953369 A	07-07-1995
		JP 8508045 T	27-08-1996
		NL 9420013 T	02-10-1995
		PL 309600 A	30-10-1995
		RU 2138257 C	27-09-1999
		SE 9502474 A	06-07-1995
WO 9836773 A	27-08-1998	AU 6436998 A	09-09-1998
CH 683920 A	15-06-1994	NONE	
WO 0024734 A	04-05-2000	AU 1324600 A	15-05-2000

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
SCB540PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 00/ 03100	07/04/2000	09/04/1999
Applicant		
ANTIBIOTICOS S.P.A.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 00/03100

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/385 A61P35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, CHEM ABS Data, MEDLINE, WPI Data, PAJ, EMBASE, BIOSIS, CANCERLIT, AIDSLINE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COLACCI A ET AL: "Inhibition of chemically induced cell transformation by lipoic acid (Meeting abstract)." PROC ANNU MEET AM ASSOC CANCER RES, (1997). VOL. 38, PP. A2419. ISSN: 0197-016X., XP000929597 Istituto Nazionale per la Ricerca sul Cancro (IST) Biotechnology Satellit Unit, Bologna, Italy 40126. the whole document --- -/--	1,4

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2000	31/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cielen, E

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
P[redacted]P 00/03100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	SILINGARDI P; NOONAN D; HORN W; VACCARI M; ARGNANI A; GRILLI S; IACONDINI A; COLACCI A : "Effect of lipoic acid on foci forming capacities of transformed cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL MEETING, vol. 39, March 1998 (1998-03), pages 289-290, XP000929579 cited in the application the whole document	1
X	US 5 679 697 A (GARNETT MERRILL) 21 October 1997 (1997-10-21) abstract column 1, line 10 - line 18 column 4, line 43 - line 65 column 5, line 14 - line 40 column 12, line 33 - line 41 column 13, line 58 -column 14, line 25 column 14, line 65 -column 15, line 19 column 15, line 59 -column 16, line 3 claims	1-4
X	WO 99 06040 A (BERRY CHRISTOPHER J ;PACKER LESTER (US); FOLEY JOHN L (US)) 11 February 1999 (1999-02-11) abstract page 1, line 5 - line 9 page 10, line 13 - line 23 page 13, line 12 - line 16 page 15, line 24 - line 28 page 16, line 26 -page 17, line 2 page 17, line 25 -page 18, line 4 page 19, line 9 - line 14 page 30, line 7 - line 13 claim 1	1,2,4
X	WO 95 13061 A (IMMUNAL KFT ;KULCSAR GYULA (HU)) 18 May 1995 (1995-05-18) abstract page 3, line 13 - line 21 page 4, line 10 - line 20 page 5, line 1 - line 29 page 6, line 1 - line 5 page 16 page 22, line 1 - line 5 claims 1,4,7,9,16,18-30	1-4

-/--

1

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 00/03100

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 36773 A (UNIV YALE) 27 August 1998 (1998-08-27) abstract page 3, line 16 -page 4, line 3 page 4, line 19 - line 27 page 5, line 6 - line 9 page 13, line 1 - line 9 page 15, line 19 -page 16, line 6 page 23, line 24 - line 28 page 25, line 20 - line 23 table 1 claims	1-4
X	CH 683 920 A (MARIGEN SA) 15 June 1994 (1994-06-15) cited in the application abstract page 2, line 1 - line 30 page 4, line 60 -page 5, line 8 page 5, line 41 - line 44 page 15, line 54 - line 56 page 16, line 13 - line 27 page 21, line 30 - line 65 claims	1,2,4
E	WO 00 24734 A (UNIV NEW YORK) 4 May 2000 (2000-05-04) abstract page 1, line 10 - line 16 page 5, line 1 - line 25 page 6, line 19 - line 35 page 15, line 9 - line 32 page 17, line 29 -page 18, line 2 page 19, line 5 - line 20	1-4

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/03100

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 5679697	A	21-10-1997	US	5463093	A	31-10-1995
			AU	1180795	A	13-06-1995
			CA	2176603	A	01-06-1995
			EP	0730449	A	11-09-1996
			WO	9514466	A	01-06-1995
			US	5776973	A	07-07-1998
WO 9906040	A	11-02-1999	AU	8768098	A	22-02-1999
WO 9513061	A	18-05-1995	HU	213677	B	29-12-1997
			AU	682735	B	16-10-1997
			AU	1074995	A	29-05-1995
			CA	2151826	A	18-05-1995
			CH	686867	A	31-07-1996
			CN	1116406	A	07-02-1996
			CZ	9501773	A	17-01-1996
			DE	4498692	T	22-02-1996
			EP	0679081	A	02-11-1995
			ES	2094702	A	16-01-1997
			FI	953369	A	07-07-1995
			JP	8508045	T	27-08-1996
			NL	9420013	T	02-10-1995
			PL	309600	A	30-10-1995
			RU	2138257	C	27-09-1999
			SE	9502474	A	06-07-1995
WO 9836773	A	27-08-1998	AU	6436998	A	09-09-1998
CH 683920	A	15-06-1994	NONE			
WO 0024734	A	04-05-2000	AU	1324600	A	15-05-2000

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir[illegible]l Application No
PCT/[illegible]00/03100

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 5679697	A	21-10-1997	US	5463093 A	31-10-1995
			AU	1180795 A	13-06-1995
			CA	2176603 A	01-06-1995
			EP	0730449 A	11-09-1996
			WO	9514466 A	01-06-1995
			US	5776973 A	07-07-1998
WO 9906040	A	11-02-1999	AU	8768098 A	22-02-1999
WO 9513061	A	18-05-1995	HU	213677 B	29-12-1997
			AU	682735 B	16-10-1997
			AU	1074995 A	29-05-1995
			CA	2151826 A	18-05-1995
			CH	686867 A	31-07-1996
			CN	1116406 A	07-02-1996
			CZ	9501773 A	17-01-1996
			DE	4498692 T	22-02-1996
			EP	0679081 A	02-11-1995
			ES	2094702 A	16-01-1997
			FI	953369 A	07-07-1995
			JP	8508045 T	27-08-1996
			NL	9420013 T	02-10-1995
			PL	309600 A	30-10-1995
			RU	2138257 C	27-09-1999
			SE	9502474 A	06-07-1995
WO 9836773	A	27-08-1998	AU	6436998 A	09-09-1998
CH 683920	A	15-06-1994	NONE		
WO 0024734	A	04-05-2000	AU	1324600 A	15-05-2000

CLAIMS

1. The use of alpha lipoic acid or physiologically equivalent derivatives thereof for the preparation of antimetastatic medicaments.

2. The use as claimed in claim 1 wherein the physiologically equivalents derivatives of lipoic acid are selected from salts, esters or inclusion complexes.

3. The use as claimed in claim 2 wherein the lipoic acid derivative is a pharmaceutically acceptable salt.

4. The use as claimed in [any one of] claim[s] 1[-3] for the preparation of antimetastatic medicaments which can be administered through the oral, intravenous or subcutaneous routes.